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Antifungal Activity of Local Microbial Isolates against Snap Bean Pathogens

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A B S T R A C T

Production of snap beans is constrained by diseases that make farmers use synthetic pesticides excessively. Use of synthetic pesticides leads to contamination of produce, threat to beneficial organisms and pollutes the environment. Microbial pesticides are eco-friendly, target-pest specific, biodegradable and safe hence alternative to synthetic pesticides. *In vitro* studies were conducted to evaluate the antifungal activity of microorganisms isolated from local environment. Test plant pathogens were isolated from diseased tissues and antifungal activity of the microbial antagonists evaluated against species of *Fusarium, Colletotrichum, Alternaria* and *Rhizoctonia* using the dual culture method. Growth inhibition was determined as reduction in plant pathogen colony diameter. A total of 42 microbial isolates were found to possess antimicrobial activity out of which 16 were most active. The most efficacious antagonists were identified as *Trichoderma viride, T. harzanium, T. asperellum* and *Paecilomyces*. *T. harzianum* was the most active and inhibited the test pathogens by up to 66%. The results indicated that local soils, rhizosphere and dead organic substrates are potential sources of microbial antagonists that can be exploited. These microbial biopesticides would serve as alternatives to synthetic pesticides in management of diseases in snap beans.

Introduction

Small holder farmers of snap beans face the constraint of diseases that affect the crop from planting to harvesting. These diseases range from root rots, wilts, stem rots, blights, leaf spots, mildews and rusts. The notable causal agents of these diseases include species of *Alternaria, Rhizoctonia, Fusarium, Colletotrichum* and *Phaeoisariopsis* (Infonet-Biovision, 2016). These economically important pathogens of snap beans lead to low yields and further damage market preferred pod quality characteristics (Mohammed, 2013; Mulanya, 2014; Nyasetia, 2011). Frequent use of synthetic pesticides in managing diseases in snap beans is unsustainable as they are hazardous to non-target organisms, pollutes the environment, lead to resistance by pathogens against fungicides and lead to chemical residues above recommended levels on pods which lead to interception and rejection of produce at export markets

(Fening *et al.*, 2014; Nyasetia, 2011; Ouma *et al.*, 2014). The strict maximum residue level (MRLs) requirements have resulted into low volumes of snap beans designated for export market because smallholder farmers need to invest heavily and still fear being non-compliant (Ouma *et al.*, 2014). Therefore, use of locally developed microbial biopesticides is an alternative to synthetic pesticides in management of crop diseases (Belete *et al*., 2015; Srinivasan, 2012).

Use of biopesticides has gained popularity all over the world and formulations are available in market as one of the alternatives of disease management (Chandler *et al*., 2011; Ouma *et al.*, 2014; Srinivasan, 2012). In Kenya commercial formulated biopesticides are of foreign origin that makes them expensive hence limiting their use by smallholder farmers. The attributes that make biopesticides to be best alternatives to synthetic pesticides include having no toxic residues, are harmless to beneficial organisms and pose minimal risk to the environment and humans, are effective as the synthetic pesticides in managing diseases and have high compatibility with other pest management techniques (Chandler *et al*., 2011; Ouma *et al*., 2014; Sola *et al*., 2014). Many species of antagonistic microorganisms inhibit mycelia growth of plant pathogens in *in vitro* bioassays (Belete *et al*., 2015; Figueirêdo *et al*., 2010; Omar and Ahmed, 2014).This implies that with relevant technology for mass multiplication and formulation, the adoption of local microbial biopesticides is relatively cheap and safer to producers, consumers and environment. The stringent requirements during snap beans marketing are remediated by use of biopesticides that present premium value snap beans on niche markets that will enable the smallholder farmers' worth in investing in snap bean production (Kimani, 2014; Ouma *et al*., 2014).This study evaluated activity of antagonistic microorganisms isolated from local environments in reducing the growth of plant pathogenic fungi *in vitro*.

Materials and Methods

Isolation of antagonistic microorganisms

Microorganisms were collected from the rhizosphere in cultivated fields and pasture land, roots, cattle shed, decaying wood and decomposing organic matter. Soils were taken at a depth of 10 cm and air-dried for seven days by spreading on surface sterilized bench. Microorganisms from soil and compost were isolated by serial dilution (Belete *et al*., 2015). Ten grams of each composite soil sample was suspended in 100 ml sterile distilled water to obtain a suspension that was serially diluted. One millilitre aliquot of each of 10^{-2}, 10^{-3} and 10^{-4} dilutions was pipetted into plates that were later inoculated with 20 ml Nutrient Agar (NA) and in triplicate. The suspension and molten media in the plates were thoroughly mixed by swirling in a gentle manner to uniformly spread the suspension under aseptic conditions.

The isolation of microorganisms from roots and decaying woods was by direct plating. The decaying pieces of wood were washed with 70% alcohol for five minutes and then cut into 3mm blocks. Three pieces of wood were direct plated in molten PDA and NA. Incubation of bacterial cultures was for 48 hours with Petri plates in inverted position under room temperature (23 ± 2 ºC). Fungal and actinomycetous cultures were incubated for seven days under room temperature (23 ± 2 ºC). After incubation, microorganisms showing clear zones of inhibition against other microorganisms were sub-cultured and purified on PDA for fungi and

actinomycetes, and bacteria on NA. Purified cultures of bacteria were inoculated on sucrose-peptone agar slants while the fungi and actinomycetes were maintained on PDA slants at 4ºC.

Isolation of plant pathogenic fungi

Fungal plant pathogens were isolated from diseased roots, stems, leaves and pods of snap beans by direct plating. The diseased tissues were first washed in running water to remove surface soil, dust and other contaminants. Small pieces measuring 3 mm in length of plant tissues were cut using sterile surgical blade and then surface sterilized in 1.3 % sodium hypochlorite for two minutes. The plant tissues were rinsed three times in sterile distilled water and then blot dried using sterile absorbent paper. Four pieces were aseptically direct plated on PDA media and incubated for seven days. Sub-culturing was done by aseptically cutting small pieces of mycelia at edges of colonies and transferring to new PDA media to make pure cultures (Siameto *et al*., 2010).

Screening and evaluation of microbial antagonists

Fusarium solani, Colletotrichum lindemuthianum and *Rhizoctonia solani* were used as test pathogens in screening of microbial isolates for antifungal activity. The agar discs having the antagonist microorganism were aseptically inoculated at four equidistant positions on PDA. Discs having six days old cultures of each plant pathogenic fungus were inoculated at the centre of the cultured plates. The control plates included test pathogens inoculated alone at the centre and the experiment was replicated three times. The plates were incubated at room temperature (23 ± 2 ºC) to allow adequate antagonist-pathogen interaction to take place. The plates were arranged in a completely randomized design. Data was collected 9 days after inoculation (DAI) on PDA media by recording the growth diameters of the pathogens in millimetres. Colony diameters of the fungal pathogens in the test and control plates were measured and recorded. The colony diameter was given as the mean of two perpendicular diameters. The data was used to obtain the inhibition in the mycelia growth of three test pathogens by the potential antagonist microbial isolates. The antifungal activity was determined by measuring the pathogen colony diameters and percentage inhibition calculated according to Silliman *et al.* (2015):

$$\text{Percentageinhibition} = \frac{A - B}{A} \times 100$$

Where A is the diameter of mycelia growth of pathogenic fungus in control and B is the diameter of mycelia growth of pathogenic fungus with antagonist.

The percentage of growth inhibition of each test pathogen by potential microorganism isolates was calculated and average percentage growth inhibition was used in rating effectiveness of isolates. Potential antagonistic microorganism isolates were categorized as effective in inhibiting radial growth of test pathogens by giving them a score as per modified Bogumił *et al.* (2013). Where; 1 – Low antagonistic activity (I < 51%), 2 – Moderate antagonistic activity (I = 51-59%), 3 – High antagonistic activity (I = 60-75%), and 4 – Very high antagonistic activity (I > 75%).

After screening, active antagonistic fungi that were rated 4 and 3 were identified. Each isolate was cultured on PDA and identified based on their colony appearance, shape of conidia and conidiophores and branching pattern of phialides. The morphological and microscopic authenticity of the antagonistic fungi was confirmed using different

identification keys (Watanabe, 2010). Evaluation of most active microbial antagonists based on results obtained after screening against *Alternaria solani, Fusarium solani, Colletotrichum lindemuthianum* and *Rhizoctonia solani* was carried out using the same protocol as screening and data was collected over time at two days interval.

Data analysis

Data on percentage colony diameter inhibition was subjected to analysis of variance using Genstat®, Release15.1. Mean separation of the treatments was accomplished using Fisher's protected Least Significant Difference (Steel *et al.*, 1999).

Results and Discussion

A total of 42 microorganism isolates showed clear zones of inhibition against other microorganisms comprised of fungi, actinomycetes and bacteria of which fungal isolates were the majority. Fungal isolates were 29, bacterial isolates were eight while actinomycetous isolates were five (Tables 1a; 1b). Four microorganism isolates had very high degree of antagonistic activity. The four isolates were from diverse sources including: milking shade mud, nappier grass rhizosphere, compost soil and decomposing wood (Table 1a). The mean percentage growth inhibitions exhibited by the four fungi were 80.4%, 79.7%, 77.7% and 77.2%. A total of 12 fungal isolates had high degree of antagonistic activity. All the 16 antagonist microorganism isolates were fungi, with genus *Trichoderma* being predominant (Table 1a). Other genus included *Paecilomyces, Epicoccum, Rhizoctonia, Sepedonium* and *Gloeosporium.*

The antagonistic fungi significantly ($P \leq 0.05$) reduced colony diameters of all the test plant pathogens. Antagonistic fungi inhibited mycelia growth of the four test plant pathogens by up to 65.8 % and 49% in experiment one and experiment two, respectively (Tables 2). In experiment one, *Trichoderma harzianum* was the most effective in reducing mycelia growth of test plant pathogens by up to 65.8% followed by *T. viride* (65.3%) while least growth inhibition was observed in *Sepedonium* (37.4%). The plant pathogens varied in their sensitivity to the different antagonistic fungi. *Rhizoctonia solani* was the most sensitive while *Alternaria solani* was the least to the activity of antagonistic fungi. *Trichoderma atroviride, Paecilomyces, Trichoderma viride* and *Trichoderma* isolate 3 had the highest biocontrol potency against *Alternaria solani, Rhizoctonia solani, Fusarium solani* f.sp. *phaseoli* and *Colletotrichum lindumuthianum,* respectively. In experiment two, *Trichoderma* isolate 1 and *Trichoderma harzianum* were the most effective and showed significant superiority amongst all the antagonistic fungi. *Rhizoctonia* was least in effectiveness in inhibiting growth of plant fungal pathogens in experiment two compared to experiment one (Tables 2; 3).

Microorganisms isolated from local sources had antagonistic activity against *Fusarium solani, Colletotrichum lindemuthianum* and *Rhizoctonia solani.* A considerable variation was depicted among, as well as within the fungal, bacterial and actinomycetous antagonists with regard to the colony diameter reduction of the three test pathogens at the ninth DAI. Similar findings were reported by Lahlali and Hijri (2010) that antagonistic fungi had significant antagonistic activity against *Rhizoctonia solani* in *in vitro* bioassay. In another study, similar findings were reported on microbial antagonists exhibiting varying degree of

antagonistic effect against *Alternaria* sp., and *Fusarium oxysporum* on PDA medium (Sivanantham *et al.,* 2013).The lumping together of all groups of microorganisms did not yield satisfactory comparative results Some bacteria had pronounced inhibition zones though the diameters of mycelia of test plant pathogens were bigger relative to most fungal isolates hence regarded to have lower antifungal activity. The slight inconsistency of results across the two *in vitro* bioassays could be attributed to sub-culturing, storage time in refrigerator of microorganism isolates and further compounded by room temperature fluctuations (Suprapta, 2012). The same variables could have been the cause of shifts in degree of activity among the microorganism isolates. The difference in response to antagonistic activity by the four test pathogens was the result of integrity of the cell wall exhibited by these pathogens.

The different *Trichoderma* spp. varied in antagonistic activity against different fungi as reported by Muthukumar *et al.,* (2011) Ramzan *et al.,* (2014), Reddy *et al*., (2014), Belete *et al.,* (2015) and El-Naggar *et al.,* (2016).The results herein were also in line with those by Reddy *et al.,* (2014) who reported that all the isolates of *Trichoderma* spp. were highly efficacious on the growth of *Fusarium oxysporum* f. sp. *lycopersici*, *Alternaria solani* and others in *in vitro* bioassay.

Table.1a Growth inhibition of three test plant pathogens by microbial isolates from diverse sources in dual culture test (Bogumil ranking)

Isolate designation	Source of collection	Group	% GI at 9th DAI			Mean	Bogumil ranking
			FO	CO	RO		
FKE3	Milking shade mud, UK	Fungi	76.4	92.1	72.6	80.4	4
FKF1	Nappier rhizosphere, UK	Fungi	76.4	92.5	70.1	79.7	4
FS4	Field Station compost, UK	Fungi	71.8	94.4	67.0	77.7	4
WOOD	Decomposing wood, UK	Fungi	67.4	83.7	80.5	77.2	4
B	Busia soil, PPL	Fungi	73.3	76.4	75.0	74.9	3
WRC(P)	Busia soil, PPL	Fungi	74.0	81.3	67.4	74.2	3
LH1K8D23	Culture, PPL	Fungi	67.5	79.0	70.9	72.5	3
FKE1	Milking shade mud, UK	Fungi	67.4	80.2	67.1	71.6	3
FKE2	Milking shade mud, UK	Fungi	66.3	72.6	72.4	70.4	3
DRC(M)	Busia soil, PPL	Fungi	64.1	71.0	65.3	66.8	3
LRC(M)	Busia soil, PPL	Fungi	63.5	78.2	67.0	69.6	3
DRC(C)	Busia soil, PPL	Fungi	61.9	83.3	63.2	69.5	3
A	Busia soil, PPL	Fungi	60.8	74.0	72.3	69.0	3
FKA2	Animal shade, UK	Fungi	59.2	65.8	56.7	60.6	3
DD2	Culture, PPL	Fungi	61.3	67.2	51.7	60.1	3
LH1K1N11	Culture, PPL	Fungi	62.5	63.5	54.2	60.1	3

UK; Upper Kabete Campus, PPL; Plant Pathology Laboratory, GI; Growth inhibition, DAI; Days after inoculation, FO; *Fusarium solani*, CO; *Colletotrichum lindemuthianum* and RO; *Rhizoctonia solani*. >75%- Very high and 60-75%- High degree of antagonistic activity.

Table.1b Inhibition of growth of three test plant pathogens by microbial isolates from diverse sources in dual culture test (Bogumil ranking)

Isolate designation	Source of collection	Group	% GI at 9th DAI			Mean	Bogumil ranking
			FO	CO	RO		
FKA3	Animal shade, UK	Fungi	62.8	60.4	54.2	59.1	2
MAIZE	Maize roots	Fungi	52.3	69.6	50.0	57.3	2
D82	Culture, PPL	Fungi	54.6	66.7	48.8	56.7	2
BEANFS	Bean roots, UK	Fungi	55.7	60.4	52.7	56.3	2
FS1	Potato rhizosphere, UK	Bacteria	55.0	67.1	45.1	55.7	2
FKB1	Bean rhizosphere, UK	Fungi	53.9	57.9	50.2	54.0	2
WRC(C)	Busia soil, PPL	Fungi	43.8	63.5	48.7	52.0	2
CN	Compost, Ndumbuini	Fungi	49.4	58.7	46.9	51.7	2
C	Lantana rhizosphere, UK	Bacteria	61.4	65.6	28.2	51.7	2
PO	Culture, PPL	Fungi	50.0	55.2	46.3	50.5	2
LH19N1	Culture, PPL	Fungi	48.0	58.3	41.2	49.2	1
FKB	Bean rhizosphere, UK	Fungi	42.4	59.1	45.8	49.1	1
FKD	Compost, UK	Fungi	42.4	42.5	58.7	47.9	1
LK69	Busia soil, PPL	Actinomycete	47.5	56.3	40.0	47.9	1
ID3	Culture, PPL	Fungi	45.4	59.1	35.8	46.8	1
FKB2	Bean rhizosphere, UK	Bacteria	36.6	57.5	39.1	44.4	1
FSL13	Silt soil, UK	Bacteria	43.0	63.1	26.6	44.2	1
AS6	Busia soil, PPL	Actinomycete	38.5	56.3	37.3	44.0	1
DRC(M1)	Busia soil, PPL	Bacteria	33.9	54.0	34.2	40.7	1
LHIK7D22	Culture, PPL	Fungi	41.5	46.4	31.3	39.7	1
WRC(M)	Busia soil, PPL	Actinomycete	39.2	42.9	28.1	36.7	1
KSC14(W)	Culture, PPL	Bacteria	29.4	44.4	32.8	35.5	1
KSC14	Culture, PPL	Actinomycete	20.6	0.0	26.9	15.8	1
AS 7	Potato rhizosphere, UK	Bacteria	10.4	0.0	20.7	10.4	1
BN	Bean rhizosphere, UK	Fungi	5.1	0.4	17.0	7.5	1
KSC14	Field Station compost, UK	Bacteria	11.0	0.0	2.3	4.4	1

UK; Upper Kabete Campus, PPL; Plant Pathology Laboratory, GI; Growth inhibition, DAI; Days after inoculation, FO; *Fusarium solani*, CO; *Colletotrichum lindemuthianum* and RO; *Rhizoctonia solani*. 51-60%- Moderate and <51%- low antagonistic activity.

Table.2 Percentage colony diameter inhibition of mycelial growth of plant pathogenic fungi by antagonistic fungi, in vitro experiment 1

Antagonist	*Alternaria*	*Rhizoctonia*	*Fusarium*	*Colletotrichum*	Mean
T. harzianum	56.4 ab	69.6 ab	68.9 b	68.5 abc	65.8 a
Trichoderma 1*	52.0 bc	44.5 g	55.4 def	59.8 ef	52.9 f
T. asperellum	52.1 bc	68.6 b	68.3 b	67.1 abcd	64.0 abc
Trichoderma 2*	48.3 cd	65.1 bcd	57.0 de	58.0 f	57.1 e
Paecilomyces	56.7 ab	74.1 a	57.5 d	64.0 cde	63.1 c
T. pseudokonongii	43.7 de	54.3 ef	55.4 def	43.3 h	49.2 g
T. konongi	52.8 abc	69.3 ab	67.4 bc	63.4 he	63.2 bc
T. reseei	52.8 abc	56.5 e	54.8 def	66.1 bcd	57.5 e
T. atroviride	58.3 a	67.7 bc	53.4 ef	61.2 ef	60.2 d
T.viride	48.2 cd	70.0 ab	73.0 a	70.1 ab	65.3 ab
Trichoderma 3*	55.7 ab	63.5 cd	64.6 c	71.5 a	63.8 abc
Trichoderma 4*	43.7 de	50.0 f	43.3 h	52.4 g	47.4 gh
Epicoccum	38.9 e	42.9 g	37.4 i	36.7 i	39.0 i
Rhizoctonia	33.5 f	61.3 d	47.7 g	43.3 h	46.4 h
Sepedonium	10.9 g	53.8 ef	42.0 h	42.8 h	37.4 i
Gloeosporium	45.0 d	67.2 bc	53.0 f	68.2 abc	57.6 de
Control	0.0 h	0.0 h	0.0 j	0.0 j	0.0 j
Mean	44.0	57.6	52.9	55.1	52.4
LSD ($P \leq 0.05$)	5.3	6.0	3.6	4.2	4.8
CV (%)	7.2	6.2	4.1	4.6	4.7

Means accompanied by different letter(s) in each column are significantly different (Duncan's multiple range test, $P \leq 0.05$).

Table.3 Percentage colony diameter inhibition of mycelial growth of plant pathogenic fungi by antagonistic fungi, in vitro experiment 2

Antagonist	*Alternaria*	*Rhizoctonia*	*Fusarium*	*Colletotrichum*	Mean
T. harzianum	48.1 ab	38.8 bc	51.5 b	53.3 a	47.9 ab
Trichoderma 1	49.5 a	38.6 bcd	58.0 a	49.2 ab	48.8 a
T.asperellum	46.9 ab	38.7 bc	43.2 c	53.4 a	45.6 b
Trichoderma 2	48.3 ab	35.5 bcde	52.3 b	52.0 a	47.0 ab
Paecilomyces	51.5 a	51.2 a	43.8 c	46.7 b	48.3 a
T. pseudokononggii	37.7 d	35.5 bcde	42.7 cd	38.1 cde	38.5 de
T. konongii	39.0 cd	31.8 ef	42.2 cd	36.6 cdef	37.4 de
T. reseei	38.9 cd	36.3 bcde	39.2 cde	32.2 f	36.6 e
T. atroviride	38.6 cd	32.8 cdef	37.0 de	39.7 cd	37.0 de
T.viride	34.6 d	40.2 b	40.8 cde	38.3 cde	38.5 de
Trichoderma 3	37.5 d	40.4 b	35.4 e	39.1 cd	38.1 de
Trichoderma 4	43.7 bc	39.7 b	44.7 c	40.6 c	42.2 c
Epicoccum	28.6 e	28.2 f	29.7 f	32.8 ef	29.8 f
Rhizoctonia	16.9 f	7.4 h	16.0 g	10.8 h	12.7 h
Sepedonium	17.6 f	21.0 g	30.1 f	16.6 g	21.3 g
Gloeosporium	38.8 cd	32.7 def	52.2 b	34.6 def	39.6 d
Control	0.0g	0.0 i	0.0 h	0.0 i	0.0 i
Mean	36.2	32.3	38.7	36.1	35.9
LSD (P ≤0.05)	5.6	5.7	5.9	5.0	5.6
CV (%)	9.4	10.6	9.3	8.4	8.7

Means accompanied by different letter(s) in each column are significantly different (Duncan's multiple range test, P ≤ 0.05).

Trichoderma harzianum had the highest antagonistic activity and this was in line with the findings by Soliman *et al.,* (2015) who reported that among the antagonists tested, *T. harzianum* highly retarded the growth of *Botrytis cinerea*. Findings in the current study were consistent with findings by Muthukumar *et al.,* (2011) in regard to *Trichoderma* spp. antagonism. Contrary findings to our study were reported by (Otadoh *et al*., 2011) that *T. reesei* had the highest inhibitory effect on mycelia growth of *Fusarium oxysporum* f. sp. *Phaseoli* among all *Trichoderma* spp. tested. Findings by Mahmoud (2015) were not in line with our findings during the first bioassay of *T. harzianum* against species of *Fusarium* and *Rhizoctonia* since *Fusarium* sp. was the most sensitive. Antifungal activity of *Trichoderma* is attributed to various

mechanisms key among them being antibiosis and competition. In addition studies by Muthukumar *et al.,* (2011) and Hermosa *et al.,* (2000), indicate that *Trichoderma* spp. produce enzymes, volatile and non-volatile metabolites that degrade cell walls of pathogens and inhibit mycelia growth.

Other species that had antagonistic activity against the test pathogens included *Epicoccum, Rhizoctonia*, *Sepedonium* and *Gloeosporium.* The efficacy of *Epicoccum* in inhibiting mycelial growth of plant pathogens may be due to its production of numerous antifungal compounds such as flavipin (Madrigal and Melgarejo, 1994). *Paecilomyces* in this study showed profound effect of suppressing the four test pathogens and corroborates with the results reported by Ramzan *et al.,* (2014). In 2015, Perveen *et al.,* reported similar findings that *Paecilomyces* sp. was highly efficacious against *Sclerotium rolfsii* and *Pythium aphanidermatum* in dual culture bioassays. Biocontrol potency of *Paecilomyces* can be attributed to production of active metabolites that inhibit growth of other microorganisms.

Another attribute of *Paecilomyces* is the capability to colonise the agar surface much faster compared to the pathogen (Muhammad and Amusa, 2003). This status of microorganism antagonist–phytopathogen specificity may be as a result of differences in levels of hydrolytic enzymes produced by each species or isolate when they attack the mycelia of the pathogens (Reddy *et al*., 2014). This study advocates that hyphal interaction and parasitism are subsets of mycoparasitism that were exhibited by antagonistic fungi (Hermosa *et al.,* 2000; Howell, 2003; Reddy *et al*., 2014; Belete *et al*., 2015).

In conclusion, the results obtained from the laboratory studies illustrate how the exploitation of local environment in search of microbial antagonists against pytopathogenic fungi in vegetables is promising. Use of antagonistic fungi in management of fungal diseases was effective under *in vitro* conditions. The findings showed that all the antagonistic fungi have antifungal effect on the mycelia growth of pytopathogens with *Trichoderma* spp. and *Paecilomyces* giving more promising results than other isolates. *Trichoderma* spp. and *Paecilomyces* could be used for management of fungal diseases in snap beans grown by farmers in Kenya. Antagonistic fungi are potentially effective in management of diseases in snap beans in fields hence more studies on efficacy should be conducted. Further work on continued exploitation of local environment in search for more microbial antagonists should be undertaken. Further research to determine active ingredients of antagonistic fungi and their formulations may be of high priority in enhancing their effectiveness in field. Intensive research on integrating biocontrol agents with conventional methods to manage diseases in snap beans should be emphasized to reduce use of synthetic pesticides.

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