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Antagonistic Potential Of Selected Fungal And Bacterial Isolates From Rhizosphere Of Sugarcane Variety Co 421 Against Sporisorium Scitamineum In Kibos, Kisumu County, Kenya

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Abstract: Sugarcane smut disease caused by a fungus Sporisorium scitamineum is a limiting factor to cane production in Kenya. It is threatening the sugar industry due to its effect on cane quality and yields. Sugarcane (Saccharum officinarum L.) is known to have microbial organisms associated with its rhizosphere with potential antagonistic activity against other rhizosphere microorganisms. Little information is available on sugarcane rhizosphere microorganisms and their antagonistic potential in Kenya. The objective of this study was to evaluate the antagonistic potential of selected microbial isolates from sugar cane Variety CO 421 rhizosphere against Sporisorium scitamineum within Kibos area, in Kisumu, Kenya. Variety CO 421 was selected because it is widely adapted and grown in all sugarcane growing areas of Kenya and its breaking resistance to smut. Screening for evaluation of potential antagonism against the test organism, were done in vitro by dual culture technique, in three replicates. In vivo screening was done in five treatments and five replicates by growing single budded sugarcane setts treated with the test organism and selected potential antagonists in plastic pots with steam sterilized soil in green house and in the field in micro plots. Setts were treated with four antagonists and distilled water as control. The experimental design was a completely randomized design. Data was collected on inhibition of mycelia growth of the test pathogen and the number of smut whips per treatment. Data on inhibition and disease incidence were subjected to analysis of variance. Treatment means were separated and compared using least Significance Difference at p=0.05. Trichoderma viride and Trichoderma herzanium inhibited Sporisorium scitamineum growth by 61% and 59% in vitro and showed 20% and 27% disease incidence in vivo respectively while AJB9 (unidentified) and Pseudomonas sp. showed inhibition zones of 25.6mm and 24.3mm in vitro and 13% and 17% disease incidence in vivo respectively. The selected isolates had evident antagonistic activity against the Sporisorium scitamineum hence recommended as potential biocontrol agent for this pathogen.
Keywords: antagonistic, biocontrol,disease incidence, invitro, screening, Sporisorium scitamineum

Introduction

The rhizosphere of plants harbours many organisms that have neutral effects on the plant, but also attracts organisms that exert deleterious or beneficial effects on the plant according to a study by Deshmuk et al. (2013). Deshmuk et al. (2013) indicated that Aspergillus, Altenaria and Rhizopus species of fungi dominated the soil. Antagonists are naturally occurring organisms with traits enabling them to interfere with pathogen growth, survival, infection or plant attack (Berg et al., 2006). The interaction between soil borne pathogens and their antagonistic counterparts are of fundamental importance for plant nutrition and health (Berg et al., 2006). Antagonistic microorganisms applied to seeds before planting colonize the rhizosphere and share the area of infection of the pathogen (Ha, 2010). Many microbial antagonists have been reported against plant fungal pathogens, such as Pseudomonas fluorescens, Agrobacterium radiobacter, Bacillus subtilis, B. cereus, B. amyloliquefaciens, Trichoderma virens, Burkholderia cepacia, Saccharomyces sp, Gliocadium sp. (Suprapta, 2012). The successful control by these antagonists mainly against the diseases caused by following genera of fungi: Alternaria, Pythium, Aspergillus, Fusarium, Rhizoctonia, Phytophthora, Botrytis, Pyricularia, Gaeumannomyces and Sporosium (Adebayo and Ekpo, 2014; Suprapta, 2012; Ru and Di, 2012).

Rhizospheric microorganisms play an important role in many processes of crop production such as decomposition, mineralization, biological nitrogen fixation, denitrification and promote growth (Pisa et al., 2011). From a study by Dua and Sidhu (2012) on effectiveness of rhizosphere bacteria for control of root rot disease and improving plant growth of wheat (Triticum aestivum), antagonistic rhizosphere microbes which inhibit the growth of pathogenic microorganisms have been found to colonize the plant's rhizosphere. Pseudomonas and Bacilli bacteria were found to be predominant showing growth promoting ability and antagonistic activity of the one hundred and thirty

isolates obtained. The study has however not been done on sugarcane and under field conditions which would validate the microbe's effectiveness before adoption as biocontrol agents in commercial agriculture.

Sugarcane smut can be of epidemic proportion especially when a susceptible variety or diseased sett is planted (Nasiru and Ifenkwe, 2004). It is classified as one of the main illnesses of sugarcane and can cause total crop failure in susceptible varieties (Briceno et al., 2005). Smut significantly reduces the yield and quality of sugarcane (Ong'ala et al., 2015; Kavitha et al., 2014; Nzioki et al., 2010; Olweny et al., 2008). Yield losses of up to 38 % and 58 % have been recorded from previous research in Kenya in plant and ratoon crops respectively (Nzioki and Jamoza, 2009), 50% and 73% in India (Viswanathan et al., 2009; Nasiru and Ifenkwe, 2004).

Sporisorium scitamineum the smut pathogen can be found in the soil as spores (teliospores) however, the spores can only survive for a short time under normal soil moisture regimes. Although it has been reported in a few other members of the grass family, there are probably no important naturally occurring alternative hosts outside Saccharum species (Comstock and Lentini, 2005). Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents since the rhizosphere provides front line defense for root against attack by pathogens (Suprapta, 2012). The pathogens encounter antagonism from rhizosphere microorganisms before and during primary infection and during secondary spread (Suprapta, 2012). Most microbial antagonistic studies have been done successfully in the laboratory with challenges under field conditions due to variations in environmental conditions (Suprapta, 2012). According to Dua and Sidhu (2012) and Alimi et al. (2012), soil microorganisms may stimulate, inhibit or completely suppress growth of soil borne pathogens. However knowledge on sugarcane rhizosphere microorganisms as potential antagonists against plant pathogens is lacking especially for Kenya. Therefore the use of microbial biocontrol agents against Sporisorium scitamineum would offer an alternative disease management strategy which is economically feasible, ecologically sound, less time consuming and environmentally safe to supplement the existing control methods.the objective of this study was to investigate the antagonistic potential of selected microbial isolates from sugarcane variety CO 421 rhizosphere against Sporisorium scitamineum causing smut disease in sugarcane. It was hypothesized that fungal and bacterial isolates from the rhizosphere of sugarcane variety CO 421 had antagonistic potential against Sporisorium scitamineum.

Materials And Methods

Study site

The study was carried out at Kenya Agricultural and Livestock Research Organization - Sugar Research Institute (KALRO – SRI) headquarters, Kibos area (Kisumu, Kenya) at an altitude of 1184 a.s.l. 0^0, 34^0 latitude and 04'S 48'E longitude. Kibos has a sub humid climate, characterized by high day temperatures, cool nights and bimodal rainfall pattern. Mean annual rainfall is 1464mm, while mean daily temperature is 23^0C.The long rains start in March and end in June, while short rains start in September and end in November. Average temperature, day lengths, evaporation and radiation vary very little throughout the year (KALRO - SRI Agro - Metrological Department).

Pathogen isolation and identification

Spores of viable sugarcane smut pathogen of CO 421 variety were obtained from the Kenya Sugar Research Foundation plant pathology laboratory sourced from freshly collected culture of teliospores having more than 70% germination from naturally infected plants (plate 1). The teliospores were surface disinfected with sterile distilled water and a loop of the fungal suspension streaked on a Petri plate containing PDA with tetracycline to prevent bacterial growth and incubated at 30^0C for 10 days. Tips of the fungal mycelia were cut and recultured to obtain pure cultures (Abd et al., 2010). Identification was done macroscopically and microscopically in reference to Cappuccino and Sherman, (2008) Ellis et al. (2007), Alexopoulos et al. (2002), Williams, (2001). The identity was confirmed by comparison with the reference strain in the Sugar Research Institute laboratories in Kibos for the macroscopic and microscopic features (Plate 2).

Plate 1: smut whip on sugarcane variety CO 421

Inhibition tests

Inhibition tests were done at Kibos (KALRO-SRI) plant pathology laboratory. Inhibition of the pathogen growth by the fungal Isolates (**AJF1 - AJF16**) was carried out on PDA medium using dual culture technique as described in Paramdeep et al. (2014) and Alwathnani and Perveen (2012), where 5mm diameter mycelial plugs of pathogen and isolated fungi (test antagonist) were cut with the help of a sterilized cork borer from the edge of 5 days old culture. The 5mm plug from each test antagonist was placed 2cm inside of the periphery of different culture plates and each plate was doubly inoculated with 5 mm diameter mycelia of the pathogen and placed 5 cm opposite the test antagonist. The dual plates were incubated at 25 ± 2^0C. Control was PDA plates inoculated with pathogen only. The experiment was performed in three replicates in a complete random design. The radial growth of the test pathogen in treated and control plates were recorded after 7 days of incubation and percentage inhibition of mycelial growth of the pathogen calculated using the formula below;

I = C - T / C × 100 …… Eqn – 5 according to Alwathnani and Perveen (2012).

Where, **I** is the percent inhibition; **C** is the pathogen colony diameter (mycelia growth) in control plate and **T** is the pathogen colony diameter (mycelia growth) in treated plate.

A loopful of test antagonist bacteria culture (Bacterial isolates **AJB 1- AJB12**) was streaked 5cm away from the plug of the pathogen by line inoculation on the same dish. Zone of inhibition was recorded as the smallest distance between the fungal pathogen and the antagonist after seven days of incubation at 28 ± 2^0C (Morang et al., 2012). The Experiment was done in three replications in a completely randomized design. The isolates that rendered highest inhibition or antagonism of pathogen mycelial growth and highest zone of inhibition in the in vitro assay were selected for pot experiment in green house and micro plots in the field (Plate 3). Four isolates, two from fungi and two bacteria were selected for green house and field experiments.

Greenhouse experiment

Plastic pots of 30 × 30 cm (20 l) with holes drilled at the bottom to facilitate drainage were surface sterilized with sodium hypochlorite and filled with steam sterilized top soil from where sugarcane has not been grown for the past five years. One budded sets of CO 421 variety were surface sterilized and dipped for 30 minutes in suspension of smut spores(4g spores/litre of sterile water) from freshly collected culture of teliospores having more than 70% germination and then held overnight in polythene sacks (Paramdeep et al., 2014; Nzioki and Jamoza, 2009; Olweny et al., 2008). Plates of antagonists cultures were grown on PDA at 25^0C for 14 days and nutrient agar medium for 2 days were scrapped using a sterilized spatula and mixed with sterilized distilled water, filtered through a nylon mesh then adjusted to get a final cfu of 1.0×10^6 spores/ml and 1.0×10^4 cfu/ml respectively is using a modified calibrated microscope slide (haemocytometer).The suspension was pippeted into the counting chambers of the haemocytometer using a clean pipette tip and the cell or spores counted in the grids of known volume (0.1µl) under a microscope to be used to estimate its concentration per ml.The smut infected one budded setts were dip inoculated for 15 minutes in the respective antagonist suspensions and the control setts dipped into sterile distilled water for similar duration.

Three setts were then planted individually in each pot and irrigated regularly, after every two days up to the second month where they were exposed to water stress to enhance development of smut fungi in the vascular system and produce symptoms.

The treatments were:

(i) Sett treatment with antagonist 1(Fungi/AJF7) + pathogen.
(ii) Sett treatment with antagonist 2 (Fungi/AJF8) + pathogen.
(iii) Sett treatment with antagonist 3 (Bacteria/AJB4) + pathogen.
(iv) Sett treatment with antagonist 4 (Bacteria/AJB9) + pathogen.
(v) Sett treatment with distilled water + pathogen (control).

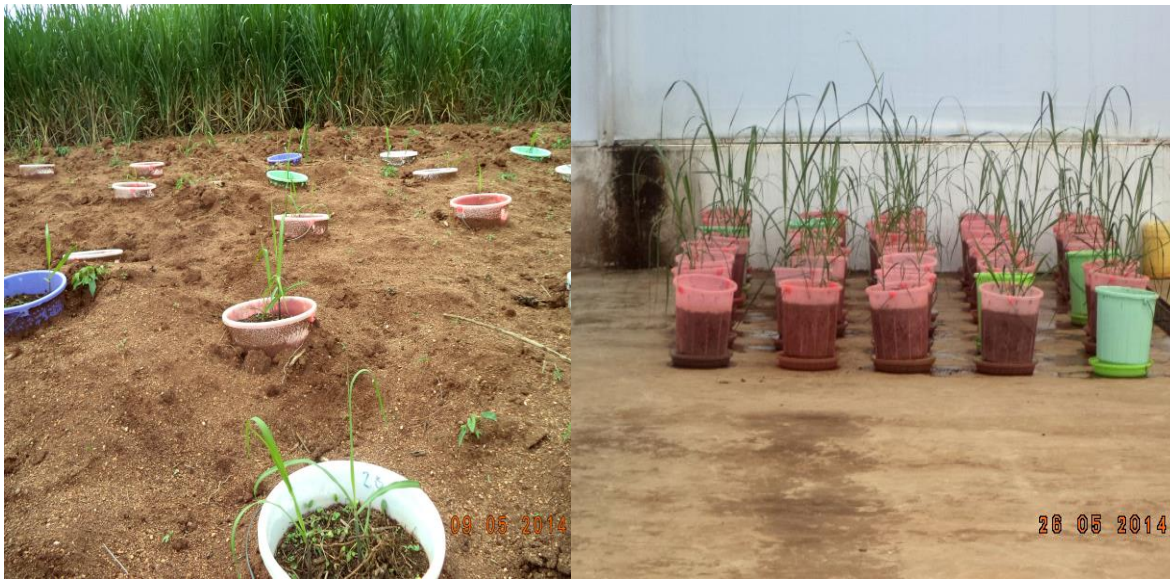
The treatments were in five replicates and the pots were arranged in a glass house in a completely randomized design. Data was collected on smut disease incidence on all treatments and control by scoring the number of smut whips appearing per treatment monthly for six months starting from two months after planting. Plants with smut whips were recorded, whip removed until the trial was completed. The data was then converted into disease incidence (DI) per treatment according to Dua and Sidhu (2012) and Morang et al. (2012) using the formulas below;

$$\textbf{(i) DI} = \frac{\textbf{Total no. of diseased plants.}}{\textbf{Total no. of observed plants}} \times \textbf{100} \text{ ... Eqn. - 6}$$

Where DI is disease incidence.

Field Experiment in microplots

This trial was established in field 7 at (KESREF), Kibos which has been well isolated to limit spread of the innoculum to commercial sugar fields. Plastic pots of 30 × 30 cm were surface sterilized with sodium hypochlorite and filled with top soil from where sugarcane has not been grown for the past five years. One budded sets of CO 421 variety were surface sterilized and dipped for 30 minutes in suspension of smut spores containing 4grams spores/litre of sterile water then held overnight in polythene sacks (Nzioki et al., 2010; Olweny et al., 2008). The treatments were laid out as in the green house in five replicates and in a completely randomized design, but the pots were buried three quarters underground in the soil to constitute a micro plot and relied on rainfall water (Plate 3). Data was collected on smut disease incidence on all treatments and control as in green house experiment above.



a. **b.**

Plate 3: Microplot (a) and green house (b) layout

Data analysis

Statistical analysis of data was conducted using SAS 9.1 package to determine the effect of antagonists on radial growth of pathogen mycelia, inhibition of growth percentage of the pathogen mycelia and smut disease incidence in the green house and field. Treatment means separation was accomplished by Turkey LSD and significance level tested at P= 0.05.

Results

In vitro screening for potential antagonism by dual culture technique

Fungi

The pathogen colony radial growth in the dual plates ranged between 12.3mm - 30mm and percentage inhibition ranged between 6 % - 61% (Table 1.1). The tested antagonists inhibited radial colony growth of the pathogen Sporisorium scitamineum at varying degrees. Isolate AJF 7 (Trichoderma viride) showed the lowest radial growth (12.3mm) and highest growth inhibition percentage of the pathogen (61%) while AJF 15 (unidentified) showed the lowest growth inhibition of 6% and highest radial growth of the pathogen (30mm). The results produced by the test antagonists were significantly different from control except AJF 15 that was not significant at (P=0.05). There was no significant difference between isolates AJF 7, 8, 9, 10 and 3 which were the best potential fungal antagonists under the conditions of this study followed by isolates AJF 1, 2, 4, 5, 6, 13, 14 and 16 were not significantly different from each other on suppression of radial growth of the pathogen (Table 1.1). The test antagonists grew faster occupying more space and showing dominance over the pathogen Sporisorium scitamineum in dual culture plates thereby limiting its growth in most of the petri plates. Isolate AJF 3, 7, 8, 9 and 10 almost completely covered the growth of the pathogen within seven days of incubation. Isolates 1, 2, 4, 5, 6, 13, and 16 inhibited the pathogen growth at varying levels. Isolate AJF 15 was dominated by the pathogen.

Table 1.1: Radial growth and inhibition percentage of pathogen Sporisorium scitamineum in dual culture plates with fungal test antagonists.

Fungal test antagonists and pathogen	Radial growth (mm)	Growth inhibition %
Control	32a	-
AJF15	30ab	6fg
AJF12	28.3b	11.3f
AJF14	25c	25.3e
AJF5	23.3cd	27e
AJF13	23cd	28.3de
AJF6	21.3de	33.3cde
AJF4	20ef	37.7bcd
AJF1	19.7ef	38.7bc
AJF16	19ef	40.7bc
AJF11	18.7ef	41.7bc
AJF2	18f	43.7b
AJF3	14g	56a
AJF10	14g	56a
AJF9	13.7g	57a
AJF8	13g	59a
AJF7	12.3g	61a
LSD	2.9	9.3

Means followed by different letters down the columns differ significantly (P=0.05). Values presented are means of three replicates.

Bacteria

The inhibition zones in dual culture plates on solid NA medium of bacterial isolates and pathogen Sporisorium scitamineum ranged from 1mm – 25.6mm after seven days (Table 1.2). The test antagonists inhibited growth of the pathogen at varying degrees. AJB 9 showed the highest inhibition zone of 25.7mm and AJF 11 the lowest zone of 1mm.The results produced by the test antagonists were significantly different from control except AJB 1, 2, 5, 7 and 11 at (P=0.05). There was no significant difference between isolates AJB 9 and AJB 4 which showed the highest inhibition followed by isolates AJB3, AJB4, AJB8, and AJB 12 that were not significantly different from each other. The test antagonists significantly reduced the growth of the pathogen except AJB1, 2, 7, 5 and 11. The antagonists grew faster than the pathogen and produced inhibition zones at varying levels there by limiting the growth of the pathogen under the conditions of this study.

Table 1.2: Inhibition zones between bacterial isolates and pathogen Sporisorium scitamineum using line inoculation method in a dual culture plate at seven days.

Bacterial isolates and pathogen	Inhibition zone (mm)
AJB9	25.7a
AJB4	24.3ab
AJB3	23abc
AJB8	23abc
AJB12	22.3bc
AJB6	21.3c
AJB10	21bc
AJB5	20.3cd
AJB7	20.3cd
AJB1	20.3cd
CONTROL	18ed
AJB2	16e
AJB11	1f
LSD	2.9

Means followed by different letters down the column differ significantly (P = 0.05).
Values presented are means of three replicates

In vivo screening for potential antagonism

AJF 7 (Trichodema viride), AJF 8 (Trichodrema herzanium), AJB 4 (Pseudomonas sp.) and AJB 9 (unidentified) were selected based on their high antagonistic potential against Sporisorium scitamineum from in vitro inhibition for potential antagonism in green house and micro plots in the months of April – November 2014.

Green house

The four selected antagonists showed varying degree of disease control from number of infected plants and percentage disease incidence in the green house. Maximum disease incidence of 27% was observed in AJF 8 (Trichodrema herzanium) and minimum of 13% in AJB9 (unidentified) in the green house compared to 30% incidence of control (Table 1.3). There was no significant difference at P=0.05 on the number of smut whips, infected plants and percentage disease incidence between the treatments.

Table 1.3: Percentage disease incidence, infected plants and number of smut whips produced by sugarcane plants treated by selected antagonists and grown under green house conditions.

Selected antagonist	Infected plants	Number of whips	Disease incidence (%)
Control	0.8a	2.8a	30a
AJB 4	0.4a	0.6a	17a
AJB 9	0.4a	0.4a	13a
AJF 7	0.6a	2.6a	20a
AJF 8	0.6a	1.6a	27a
LSD	0.98	2.69	33.9

Means followed by same letters down the column do not differ significantly (P = 0.05). Values presented are means of five replicates.

Field in microplots

The four selected antagonists showed varying degree of disease control in the field in micro plots from number of infected plants and percentage disease incidence (Table 1.4). Maximum disease incidence of 13.3% was observed in AJB 4 (Pseudomonas sp.) and minimum of 6.7% in AJF7 (Trichodema viride), compared to 36.6% in control. There was no significant difference at P=0.05 on percentage disease incidence, number of whips and percentage disease incidence between the treatments.

Table 1.4: Percentage disease incidence, infected plants and number of smut whips produced by sugarcane plants treated with selected antagonists and grown under field conditions.

Selected antagonist	Infected plants	Number of whips	Disease incidence (%)
Control	0.6a	3.6a	36.6a
AJB 4	0.4a	3.4a	13.2a
AJB 9	0.2a	1.4a	10a
AJF 7	0.2a	3.0a	6.7a
AJF 8	0.4a	2.2a	13.3a
LSD	0.79	6.80	35.60

Means followed by same letters down the column do not differ significantly (P = 0.05).Values presented are means of five replicates.

Discussion

In vitro screening

Results from this study revealed that the test antagonists checked the growth of the pathogen at varying degree. Fungal test antagonists grew faster than the pathogen considerably hindering its radial growth and bacterial test antagonists produced inhibition zones thereby limiting the growth of the pathogen in solid media. This indicated that the isolates had a considerable antagonistic effect against the pathogen Sporisorium scitamineum hence are potential biocontrol agents of the pathogen. The antagonistic effect of Fungi and Bacteria against plants fungal pathogens have already been reported by other investigators (Suprapta, 2012; Morang et al., 2012). Mechanisms of inhibition include mycoparasitism, antibiosis, metabolite production and competition for available nutrients as confirmed by a study done by Adebola and Amadi (2010) in Nigeria on the potential of three Aspergillus species isolated from cocoa rhizosphere and rhizoplane as biological control agents of Phytophthora palmivora in solid media and a study done by Alwathnani and Perveen (2012) on biocontrol of Fusarium wilt on tomatoes in Saudi Arabia, where P. citranum by 24.4% , A.niger, 35.6%) , T. herzanium ,44.4% and Penicillium sp. 0.9% inhibited radial growth of the test pathogen Fusarium oxyporum in vitro. Pankhurst et al. (2000) confirms that Pseudomonas sp., Bhukolderia sp. and Bacillus sp. in the rhizosphere of sugarcane showed in vitro inhibition of the growth of Pachymetra chunorhiza and Pythium graminicola plant fungal pathogens but not on Sporisorium scitamineum.

A study done by Usha et al. (2012) confirms that Aspergillus sp. and Trichoderma sp. effectively suppressed F.oxysporum causative agent of fusarial wilt in common vegetables in vitro by 42.5% and 45% respectively indicating they have antagonistic effect against the plant pathogen however the antagonists were sourced from vermicompost and not from the vegetables rhizosphere. Gawade et al. (2012) reported that different strains of Trichorderma isolated from sugarcane rhizosphere inhibited mycelia growth of Fusaruim moniliformae causing wilt disease of sugarcane in vitro in dual culture method indicating trichoderma's potential as biocontrol agent recording 41.98% inhibition of mycelia growth. The antagonistic potentiality of some soil fungi against Colletotrichum falcatum a pathogen causing Red rot

disease in sugarcane was also studied by dual culture method. The pathogen and individual species of the soil fungi showed varying degrees of percentage inhibition of mycelia growth that is Botrytis cinera 75%, Penicillium chrysogenum 69%, P.citrinum 23%, Gliocladium virens 56%, Trichoderma glaucum 72%, T.harzianum 53%, T.koeningii 73% and T.viride 70% grown on PDA medium (Prince et al., 2011).

The varying degrees of antifungal activity in the various studies could be due to differences in environmental parameters, media and assay methods (Prince et al., 2011). The degree of effectiveness of the various antagonists varied according to the nature, quality and quantity of the inhibitory substances secreted by the antagonists (Alwathnani and Perveen, 2012). The results of this study were confirmed by a study done by Paramdeep et al. (2014) on management of smut Sporisorium scitamineum with fungicides and bioagents invitro where Trichoderma herzanium showed mycoparasitsm and completely covered the growth of the pathogen in 7 days.

In vivo screening

All the selected four potential antagonists invitro showed varied percentage of smut disease incidence and suppression of smut whip occurrence however there was no significant difference between the treatments in the green house and also between the treatments in the field indicating none was superior to the other in vivo. There was no significant difference (p=0.05) between the treatments and control suggesting that the selected potential antagonistic microorganisms failed to effectively control sugarcane smut infection both in the field and green house. This was probably due to variations in environmental factors between the point of microbe isolation and point of action and the method of antagonist application which often leads to failure in vivo as observed by Suprapta (2012). These findings are in accordance to an investigation done by Paramdeep et al. (2014) that recorded no significant difference between Trichoderma herzanium (49.99%) a bioagent treatment and 53.36% of control treatment for smut disease incidence. Meena and Ramyabharathi (2012) also recorded 8.1% sugarcane smut disease incidence against 20% of control which indicated non effectiveness of the bioagents (Pseudomonas fluorescence and a combination of Trichoderma viride, Bacillus subtilis and Pseudomonas fluorescence) in reducing smut disease infection. Lal et al. (2009) observed 8.89% smut disease incidence against 15.1% of control with Trichorderma viride.

In general, the potential antagonistic microorganisms selected from in vitro tests often fail to effectively control plant disease in vivo (greenhouse or field trials), particularly to soil borne pathogens, this is attributed to several factors such as the type and the content of organic matter, pH, nutrient level, and moisture level of the soil that often influence the efficacy of the biocontrol agents (Suprapta, 2012). Trichoderma herzanium effectively controlled Fusarium oxyporium; Rhizoctonia solani and Alternaria alternate in chili in vivo but this was after formulation with talcum powder and applied as foliar spray. Formulation maintains the inoculation level of the bioagent while non formulation reduces the level (Subash et al., 2013) and species of Trichoderma have been shown to be selective to different fungi (Adebola and Amadi, 2012). The fungal isolates AJF 7(Trichorderma viride) and AJF 8(Trichordema herzanium) have been identified as active rhizosphere colonizers with a noteworthy effect against various plant pathogens due to their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, capacity to modify the rhizosphere, strong aggressiveness against phytopathogenic fungi and efficiency in promoting plant growth and defense mechanisms (Benitez et al., 2004). AJB 4 (Bacillus sp.) and AJB 9 (Pseudomonas sp.) mechanisms of antagonism are first rate of growth, ability to colonize the rhizosphere aggressively and ability to produce acidic substances, competition with root pathogens for nutrients and root surface colonization (Gravel et al., 2005; Pal and Mc Spadden, 2006).

Conclusions

In vitro studies on potential antagonism revealed that most of the isolated potential antagonists had evident antagonistic activity against the pathogen (Sporisorium scitamineum) that causes smut disease in sugarcane with regards to dominance and inhibition of the pathogen's mycelia growth on the dual culture plates presenting them as potential microbiological control agents against the pathogen. In vivo studies on smut disease control in the green house and micro plots revealed that the selected four potential antagonists AJF 7 (Trichoderma viride) and AJF 8 (Trichoderma herzanium) for Fungi and AJB 4 (Pseudomonas sp.) and AJB 9 (unidentified) for Bacteria failed to effectively control sugarcane smut disease both in the field and green house hence may be used as microbiological control agents against Sporisorium scitamineum subject to further studies on the best inoculation method, formulation and their ecological fitness in the actual field conditions. Isolates AJF7, AJF8, AJB4 and AJB9 may be recommended for use as potential biocontrol agents in Kibos area, Kisumu, Kenya after exploring further their performance under actual field conditions to test whether they are ecologically fit to survive, become established and function within the particular conditions of the ecosystem before their application as biocontrol agents in commercial Agriculture. Further research work is needed to investigate the best antagonistic strain in relation to time of application, inoculation method and carrier material in an effort to control Sporisorium scitamineum in vivo.

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