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ENUMERATION AND IDENTIFICATION OF RHIZOSPHERIC MICROORGANISMS OF SUGARCANE VARIETY CO 421 IN KIBOS, KISUMU COUNTY, KENYA

Juma E. O. A.[1]
Musyimi D. M.[2+]
Opande George[3]

[1,2,3]*Department of Botany, School of Physical and Biological Sciences, Maseno University Private Bag, Maseno, Kenya*
[1]*Email: tonyjuma@ymail.com Tel: +254722683893*
[2]*Email: dmusyimi@maseno.ac.ke Tel: +254703552499*
[3]*Email: opandeg@gmail.com Tel: +254715847663*

(+ Corresponding author)

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ABSTRACT

Sugarcane (*Saccharum officinarum* L.) is known to have microbial organisms associated with its rhizosphere which have potential antagonistic activity against other microorganisms. However numerous studies on rhizosphere microbial diversity have concentrated on other field crops such as rice and wheat. Little attention has been given to sugarcane. The objectives of this study were to enumerate fungi and bacteria in the rhizosphere of sugarcane variety CO 421 and identify the fungi and bacteria within rhizosphere of sugarcane variety CO 421 in Kibos, Kenya Agricultural and Livestock Research Organization – Sugar Research Institute in Kisumu, Kenya. The sugarcane Variety CO 421 was selected for this study because it is widely adapted and grown in all sugarcane growing areas of Kenya. Rhizosphere soil samples were collected randomly from ten fields of the sugarcane variety using a soil auger and trowel into sterile polythene bags. Colonies were isolated from the soil samples in three replicates, following serial dilution and plating techniques on potato dextrose agar for fungi and nutrient agar medium for bacteria. The microbes were identified under a phase contrast microscope, based on their morphological, biochemical characters, taxonomic guides and standard procedures. Data was collected on colony forming units, colony and cell morphological characteristics. Data on microbial count were subjected to analysis of variance. Field means were separated and compared using Fishers Least Significance Difference at p=0.05. Sixteen pure fungal isolates were tentatively identified and four isolates unidentified. *Trichoderma* was predominant , followed by *Aspergillus* and then *Rhizopus*, *Penicillium* and *Alternaria*. Twelve pure bacterial isolates were tentatively identified as gram negative bacteria. *Pseudomonas* was predominant, followed by *Bacillus* and *Azobacter*. The study indicated an average population of 1.30×10^7 cfu/g and 4.88×10^4 cfu/g bacteria and fungi respectively in the rhizosphere soil samples.

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a perennial grass in the family of Poaceae cultivated for its stem (cane) which is primarily used to produce sucrose (cane sugar). Sugarcane plays a major role in the economy of sugarcane growing areas worldwide. Globally it is an important source of commercial sugar accounting for nearly 70 percent of the world's sugar production [1]. Sugarcane is multipurpose crop whose other products include paper, ethanol,

animal feed, biofertilizer, alcohol derived chemicals, antibiotics, particle board and raw material for generating electricity. About twenty countries in Asia Pacific region grow sugarcane on a commercial basis [1].

The rhizosphere is an area of intense microbial activity. Exudates released by plants roots are a main food source for the microbes and a driving force for their population density and activities [2]; [3]. The population of microbes in the rhizosphere differs quantitatively and qualitatively [4]. Different plant species host specific microbial communities [5, 6]. A great majority of organisms in the rhizosphere are bacteria and fungi, actinomycetes, protozoa, microalgae and micro fauna [7]; [8]; [9]; [10]. Microbial population is stimulated in the rhizosphere by the exudates released by the plant root [6]; [11]. Bacteria has the highest stimulation followed by fungi and actinomycetes from a comparison between the number of microorganisms per gram of rhizosphere soil to the number of microorganisms per gram of a corresponding non rhizosphere soil sample [6]; [11]; [12]. These studies therefore have created the need to explore the rhizosphere microorganisms of sugarcane by unraveling their possible relationships with the sugarcane plants. The diversity and composition of the microbial taxa in the rhizosphere can be affected by several factors including plant species, soil management practices, soil type, microbial interactions and other environmental variables [11]. A study by Chandrashekar, et al. [13]; Gaddeya, et al. [14] on soil mycoflora in different crop fields of crop plants, isolated and characterized *Aspergillus*, *Penicillium*, *Trichoderma*, *Curvularia*, *Fusarium* and *Rhizopus*. The species in the fields differed in population and diversity per crop. Chandrashekar, et al. [13]; Damle and Kulkarni [15] isolated *Curvularia lunata*, *Alternaria alternate*, *Penicillium fumiculosum*, *Penicillium chrysogenum*, *Fusarium solani*, *Rhizopus stolonifer*, *Mucor* sp., *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger* from sugarcane rhizosphere. Al-Nur and Abdulmoneim [4]; Deshmukh, et al. [16] found that *Aspergillus*, *Penicillium*, *Rhizopus*, *Curvularia* and *Fusarium* were abundant on the rhizosphere mycoflora of sugarcane. Similar studies under similar conditions on soil bacteria and fungi have not been reported in Kenya. Dua and Sidhu [17]; Sood, et al. [18] studied tea rhizosphere of Indian Himalayan regions for bacterial dominance and antagonism which indicated *Bacillus* bacteria of up to 45% occurrence and *Pseudomonas* of up to 85% occurrence to dominate the rhizosphere of established and abandoned tea bushes, respectively. In a study by Angel, et al. [7]; Food and Agriculture Organization of the United Nations FAO [19] on isolation of siderosphore producing bacteria from rhizosphere soil and their antagonistic activity against selected fungal pathogens in Porur rhizosphere of tomatoes and paddy rice revealed the presence of eleven bacterial isolates which included, Fluorescent *pseudomonas*, *Bacillus*, *Azobacter* and non-fluorescent *pseudomonas* species. Gaddeya, et al. [14]; Nekade [20] isolated forty three bacterial isolates from sugarcane rhizosphere. Genera *Bacillus* was found to be the most dominant followed by *Pseudomonas*. Similar studies on sugarcane rhizosphere microorganisms in Kenya have not been reported. Cappuccino and Sherma [11]; Tamilarasi, et al. [21] in their study of diversity of root associated microorganisms of selected medicinal plants and influence of the rhizomicroorganisms on the antimicrobial property of *Coriandrum savitum* in India indicated that bacterial population was higher in the entire root zone of the plants followed by fungal and actinomycetes population. Similarly the number of microorganisms was higher in the rhizosphere soil than in the non-rhizosphere soil with greater rhizosphere effect seen in bacteria than fungi and actinomycetes. Rhizospheric microorganisms play important roles in many processes of crop production [22]. From a study by [7, 17] 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. A study by Afzal, et al. [2]; Deshmukh, et al. [16] in India isolated the largest number of fungi from the rhizosphere soil of sugarcane. The Sugarcane varieties promoted fungal development in the vicinity of the root zone. Numerous studies on rhizosphere microbial diversity and their antagonistic activity against fungal plant pathogens have focused on other crops such as rice [23] tomatoes [24] and wheat [7]. Similar studies involving the rhizospheric microorganisms are lacking for sugarcane in Kenya.The main objective of this study was to determine the population and morphologically identify microorganisms in the rhizosphere of sugarcane (*Saccharum officinarum* L.) variety CO 421 plants from Kibos area in

Kisumu County (Kenya). It was hypothesized that there were high populations of fungi, bacteria and morphologically diverse fungal and bacterial isolates in the rhizosphere of sugarcane variety CO 421. CO 421 is an imported sugarcane variety from India (Coimbatore). It has pale green stalks of medium thickness. CO 421 is a high cane and sugar yielding variety. CO 421 is of commercial importance in Western Kenya [25].

2. MATERIALS AND METHODS

2.1. Field Site Characteristics

The study was carried out at Kenya Agricultural and Livestock Research Organization - Sugar Research Institute (KALRO – SRI) headquarters, Kibos area, Kisumu in Kenya (Figure 1) 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, Table 1).

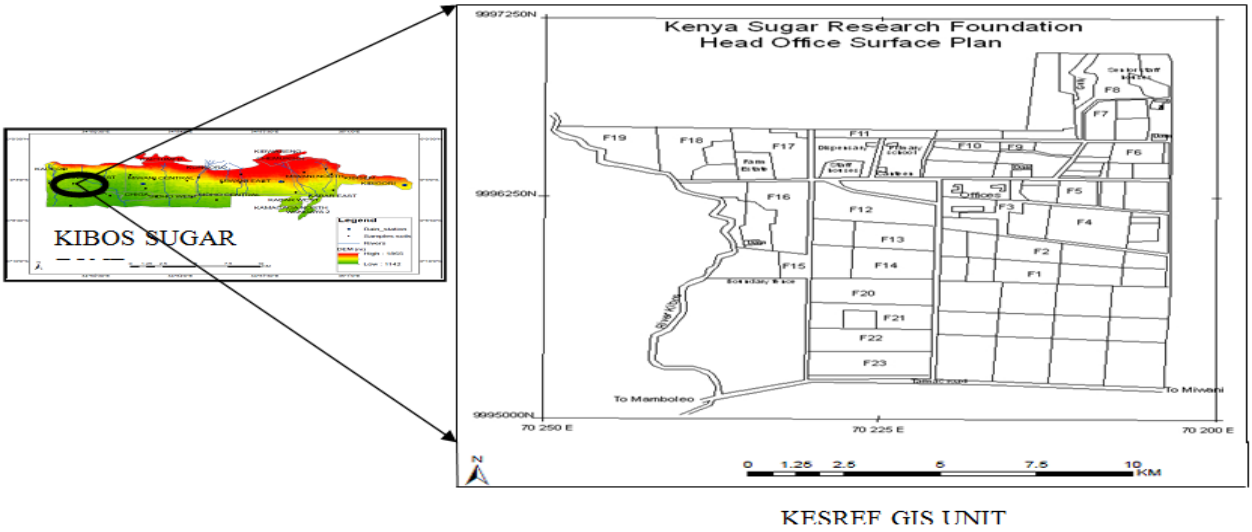

Figure-1. F1- F26 – Sugarcane Fields

Source: KESREF 2016

Table-1. Long term weather data of Kibos area

Months / Weather parameter	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Mean
Rainfall mm	97.5	85.5	155.0	207.1	107.0	67.2	74.5	112.7	111.4	101.7	138.3	106.1	1364	113.67
Evaporation (mm)	179.8	182.0	176.7	141.0	139.5	120.0	130.2	139.5	141.0	148.8	129.0	155.0	1782.5	148.54
Moisture deficit(mm)	-82.3	-96.5	-21.7	66.1	-32.5	-52.8	-55.7	-26.8	-29.6	-47.1	9.3	-48.9	-418.5	-34.88
Radiation MJ/m^2/month	858.7	910.0	880.4	852.0	756.4	720.0	747.1	771.9	807.0	864.9	801.0	871.1	9840.5	820.04
Sunshine hrs/month	269.7	257.6	226.3	201.0	145.7	183.0	204.6	220.1	195.0	201.5	195.0	241.8	2541.3	211.78
Temp max o C	31.6	30.2	28.2	26.8	28.2	27.4	25.8	25.5	26.6	27.3	27.0	27.1	331.7	27.642
Temp min o C	15.0	16.5	15.9	16.1	17.0	14.1	14.6	14.6	14.7	15.2	15.2	15.3	184.2	15.35
RH % 0900	65.7	60	72.9	74.2	77.4	75.7	64	64.1	58.6	58	68.1	66.3	80.5	67.0
RH % 1500	41.9	34.5	43.5	50.7	50.4	53.5	42.0	40.1	40.6	40.0	47.7	44.7	529.6	44.133

(**Source:** KALRO - SRI Agro - Metrological Department)

2.2. Soil Physical And Chemical Characteristics

The soils have been characterized by high clay content (over 60%), pH range of 5 – 6, high water holding capacity of 213mm/m, organic content of 0.5 - 0.75% and negligible permeability (KALRO - SRI Agro - Metrological Department).

2.3. Sampling

Sugarcane rhizosphere soil samples were collected from 10 different experimental fields with long term sugarcane cropping history (Table 1.1) at KALRO-SRI Kibos with *Saccharum officinarum* L. cultivar CO 421 between 45-315 days old. Selection of the CO 421 variety was based on the fact that it is widely adapted and grown in all sugarcane growing areas covering 28.4% of the total area under cane in Kenya, has breaking resistance to smut disease, is a good germinator and has lower rate of deterioration after maturity compared to new improved varieties hence is of commercial importance in western Kenya [26]; [27]; [25]. Soil samples were collected from five randomly chosen plants per plot at the center and the four corners along 5-25cm depth within the rhizosphere after removing top 5cm litter layer using an auger and trowel. Soil sample Collection was along the roots and the soil particles closely adhering to the roots ware transferred to sterile polythene bags with the help of a brush as described in [11, 21]. Non rhizosphere soil was also sampled corresponding to each rhizosphere soil sample with the help of a sterilized cork borer pushed horizontally to the ground same depth as in rhizosphere after removing 5cm litter layer using aseptic procedures ten centimeters away from the sugarcane root. The soil samples were emptied into sterilized polythene bags to act as control [3]. The soil samples were appropriately labeled then transported in a cool box to the plant pathology laboratory at Kibos (KALRO-SRI Headquarters) for processing.

Table-1.1. Soil samples collected from ten fields in Kibos

Composite Soil sample	Field	Sampling location
1	1	F12
2	2	F24
3	3	F17
4	4	F10
5	5	F7
6	6	F6
7	7	F4
8	8	F25
9	9	F23
10	10	F1

Source: Thesis 2015

2.4. Preparation of the Soil Samples

The five soil samples randomly collected from each field were bulked to form one composite sample by mixing thoroughly; air dried for two hours at room temperature then sieved using a 2ml mesh sieve to remove plant debris. Ten grams subsample of soil from each of the ten composite samples was used for isolation of soil microorganisms. Ten grams of non-rhizosphere soil subsample (control) was also obtained and prepared in a similar manner from each field and all the prepared samples were stored at 4°C until further analysis [15]; [28].

2.5. Determination of the Population of Fungi and Bacteria in the Sugarcane Rhizosphere Variety CO 421.

Isolation of microorganisms from the soil samples were conducted in the plant pathology laboratory at Kibos (KALRO-SRI headquarters) Kisumu, following soil dilution and plating techniques as described by Makut and Owolewa [29]; Kumar, et al. [30]; Kumalawati, et al. [28]; Shiny, et al. [31] and Chandrashekar, et al. [13]; Gaddeya, et al. [14] on different selective media and enumerated to estimate microbial population per gram of the original soil sample before sub culturing to obtain pure cultures.

2.6. Media Preparation

The following media were prepared according to manufacturer's instructions, sterilized and poured in sterilized petri dishes.

(i) Potato dextrose agar (PDA) was prepared by suspending 39.0g in 1000mls of distilled water in a conical flask, heated to boil to dissolve the media completely and sterilized by autoclaving at 15lbs pressure (121°c) for 15 minutes. (HIMEDEA Laboratories Pvt. Ltd).

(ii) Nutrient agar (NA) was prepared by suspending 28g in one litre of distilled water, heated to boil to dissolve the media completely and sterilized by autoclaving at 121°c for 15 minutes (OXOID Ltd.Basing stoke, Hampshire) according to Gowsalya, et al. [22]; Kumar, et al. [30] and Abdulkadir and Waliyu [1]; Ellis, et al. [32].

The media were well mixed before dispensing. One percent tetracycline solution was added to the PDA medium that is just above setting temperature before pouring into Petri plates to prevent bacterial growth. Fifteen milliliters of each media was transferred into sterilized disposable petri dishes, 90mm in diameter and allowed to cool under aseptic conditions in the laminar flow chamber before being used. The media were used since PDA was selective for fungi and NA for bacteria and their simple formulation. PDA medium is the most commonly used media as it is the best for mycelia growth and has a potential to support a wide range of fungal growth [29].

2.7. Isolation and Enumeration Procedure

Ten grams of soil sample was suspended in 90 ml of double distilled water to make a total of 100 ml suspension. The suspension was stirred and poured into a sterile 250 ml Erlenmeyer flask and shaken thoroughly for thirty minutes to a homogeneous solution. One ml of the suspension was pipetted aseptically and dispensed into dilution test tubes with 9 ml of sterilized distilled water to make microbial suspensions (10^{-1} to 10^{-5}). Dilutions of 10^{-2}, 10^{-3} and 10^{-4} were used to isolate fungi and bacteria in order to avoid crowding of colonies. One ml aliquot of microbial suspension of each concentration was added to sterile petri dishes containing solidified 15-20ml of sterile potato dextrose agar. Three plates were provided for each dilution (Triplicate). One percent tetracycline solution was added to the medium that is just above setting temperature before pouring into Petri plates to prevent bacterial growth. The plates were rotated by hand in a broad and slow swirling motion to disperse the soil suspension. The Petri dishes were covered, sealed with para film, turned upside down and incubated at 25 ± 2°C in the dark for daily observation up to 5-10 days for fungal growth. For bacteria 0.1 ml aliquot of microbial suspension of each concentration was added to sterile petri dishes containing solidified 15-20ml of nutrient agar medium. Sterilized bent glass rod was used to evenly spread and distribute the aliquot. Three plates were provided for each dilution and incubated at 30 ± 2°C to be observed for 2-5 day for bacterial growth after plating. The dilution with plates of countable number of colonies were selected and counted after 72 hours for fungi and 48 hours for bacteria. The number of microorganisms per gram of the original sample was calculated using the formulas;

a) **Number of microbes /ml** = $\dfrac{\text{Number of colonies (CFUs)}}{\text{Amount plated} \times \text{Dilution}}$ ..... Eqn – 1

b) **Number of microbes / gram of soil** = $\dfrac{\text{Num.} \times \text{Vol. 2}}{\text{Mass}}$ .....Eqn – 2

Where **CFU** is Colony Forming Units; **Num.** is the number of microbes/ml calculated in(a) above; **Vol. 2** is the volume of the original sample; and **mass** is mass of the solid material added to the original suspension according to Reynolds [33].

The quantitative rhizosphere effect of the plants was calculated using the formula;

R/S = $\dfrac{\text{Number of microorganisms per gram of rhizosphere soil}}{\text{Number of microorganisms per gram of non rhizosphere soil}}$ ..... Eqn – 3

According to Sule and Oyeyiola [34], Nannipieri, et al. [35] Where **R/S** is the rhizosphere effect.

2.8. Purification of Fungal and Bacterial Isolates

Morphologically different fungal colonies were selected from the petri dishes for pure culturing. Purification was done by cutting the mycelia tips with a sterile inoculating needle, transferring to a new PDA medium (sub culturing) repeatedly to obtain a pure culture [29]; [35]; [16].

Distinct individual bacterial colonies were selected from the plates and purified by streaking repeatedly on new nutrient agar plates (re-inoculation) with the aid of a sterile wire loop until all colonies were identical [30]; [20]; [36]; [37]. The pure cultures were maintained in PDA slants and plates in a refrigerator at 4^{O}C for identification and antimicrobial tests.

The percentage frequency of occurrence of each isolate was calculated using the formula;

A/B x 100…….. Eqn – 4 according to Makut and Owolewa [29]; Ong'ala, et al. [38].

Where **A** is the number of sites in which the species was observed and **B** is total number of sites**.**

2.9. Morphological Identification of Fungi and Bacteria in the Sugarcane Rhizosphere Variety CO 421

2.9.1. Fungi

Identification was done macroscopically by visual observation of petri dishes for the colony characteristics (color, shape, diameter, margin, elevation and presence of aerial mycelium) [29]; [39] and microscopic observations in slide culture, by wet mounting using lacto phenol cotton blue staining technique (LPCB) for shape, size, conidia, conidiophores and arrangement of spores according to a method described by Ibrahim and Ramha [23]; Prashar, et al. [40].

2.9.1.1. Lacto Phenol Cotton Blue Staining Procedure

A drop of the stain was placed on clean slide with the aid of a sterile mounting needle, a small portion of the mycelium from the fungal cultures was removed and placed in the drop of lacto phenol stain. The specimen was teased carefully using inoculating wire loops to avoid squashing and over-crowding of the mycelium and with the aid of the needle, a cover slip was gently applied with little pressure to eliminate air bubbles. The slide was mounted and observed with x10 and x40 objective lenses respectively under a phase contrast microscope, model: Carl zeiss. Identity was confirmed with the help of literature [37]; [41]; [42]; [43].

2.9.2. Bacteria

Identification was done microscopically by observing colony features (Surface, shape, pigmentation, margin, elevation and opacity) for characteristics that may be unique to it hence preliminary identification [31] and cell features (shape, arrangement and gram reactivity according to Kimberly and Elsa [27]; Nihorimbere, et al. [36] and Cappuccino and Sherma [11]; Nzioki, et al. [37] with reference to Bergey's manual of determinative bacteriology identification flow chart for identity confirmation.

2.9.2.1. Gram Staining Procedure

Heat fixed bacterial smear on a slide was flooded with crystal violet stain for one minute, then washed off with tap water. Gram iodine was applied for one minute and washed off with tap water. 95% alcohol was added drop by drop until it ran almost clear then washed off with tap water and counterstained with safranin and allowed 30 seconds staining then washed off with tap water, drained and blotted to dry. The slide was then examined under an oil immersion microscope for purple (G+) or pink (G-) color according to Gowsalya, et al. [22]; Kumar, et al. [30].

3. DATA ANALYSIS

Statistical analysis of data was conducted using SAS 9.1 package to determine the effect of rhizophere on microbial population. Field means separation was accomplished by Turkey LSD and significance level tested at P= 0.05.

4. RESULTS

4.1. Population of Fungi and Bacteria in the Rhizosphere of Sugarcane Variety CO 421

4.1.1. Fungal Count

There was a significant difference at P=0.05 between the populations of fungi in different fields (Table 1.2). Field ten had the highest population of fungi (6.75×10^4cfu/g) significantly different from all other fields except field 3 and field six had the least population (3.42×10^4cfu/g) significantly different from fields 2, 3, 5, 6, 7, 9 and 10. Fungal count in cfu/g of the rhizosphere and non rhizosphere soil samples of sugarcane collected and enumerated from mixed culture colonies between the month January and March 2014 showed a that rhizosphere had a higher mean value of 4.89×10^4 cfu/g of soil ranging from 3.48×10^4 to 6.48×10^4 cfu/g compared to 3.14×10^4 cfu/g of non rhizosphere ranging from 2.25×10^4 to 5.97×10^4 cfu/g making variation in population between the two regions evident. The mean rhizosphere effect was 1.7 indicating that the population in the rhizosphere was twice more than the non rhizosphere.

4.1.2. Bacterial Count

There was a significant difference between the populations of bacteria in different fields (Table 1.2). Field one had significantly different population of bacteria (2.18×10^7cfu/g) from all the other fields. Field 2 had the least population of 7.92×10^6cfu/g significantly different from fields 1, 5, 7 and 9. Bacterial count in cfu /g of the rhizosphere and non rhizosphere soil samples of sugarcane collected and enumerated from mixed culture colonies indicated that rhizosphere had a higher mean value of 1.265×10^7 cfu/g of soil ranging from 8.82×10^6cfu/g to 2.18×10^7 compared to 6.23×10^6 cfu/g of non rhizosphere ranging from 4.05×10^4 to 9.57×10^6 cfu/g of soil. The mean rhizosphere effect was 2.2 indicating that the population in the rhizosphere was twice more than the non rhizosphere. The population of bacteria in the CO 421 sugarcane variety rhizosphere was much higher than the population of fungi. Bacteria had a higher mean population of 1.27×10^7cfu/g compared to fungi's of 4.89×10^4cfu/g.

Table-1.2. Fungal and bacterial counts in the rhizosphere and non rhizosphere soil samples from ten fields of sugarcane variety CO 421.

Field	Fungi			Bacteria		
	Rhizosphere (cfu/g)	Non Rhizosphere(cfu/g)	Rhizosphere effect	Rhizosphere (cfu/g)	Non Rhizosphere (cfu/g)	Rhizosphere effect
1	3.48×10^4 e	2.25×10^4 c	1.61	2.18×10^7 a	7.02×10^6 bc	3.11
2	5.04×10^4 cd	3.18×10^4 bc	1.61	7.92×10^6 c	4.05×10^6 e	1.98
3	6.48×10^4 ab	5.97×10^4 a	1.08	1.26×10^7 bc	9.57×10^6 a	1.31
4	4.65×10^4cde	2.79×10^4 bc	1.67	1.15×10^7 bc	5.22×10^6 de	2.22
5	4.77×10^4 cd	3.15×10^4 bc	1.54	1.49×10^7 b	8.4×10^6 ab	1.79
6	3.42×10^4 e	2.49×10^4 bc	1.40	1.13×10^7 bc	4.11×10^6 e	2.79
7	5.1×10^4 cd	3.48×10^4 b	1.50	1.41×10^7 b	7.86×10^6 b	1.79
8	3.9×10^4 de	2.88×10^4 bc	1.35	9.0×10^6 c	5.43×10^6cde	1.87
9	5.28×10^4 bc	2.31×10^4 c	2.33	1.47×10^7 b	6.03×10^6 cd	2.57
10	6.75×10^4 a	2.88×10^4 bc	2.43	8.82×10^6 c	4.56×10^6 de	2.78
Mean	4.89×10^4	3.14×10^4	1.65	1.27×10^7	6.23×10^6	2.22
LSD	1.23×10^4	1.10×10^4		4.84×10^6	1.67×10^6	

Means followed by different letters down the columns differ significantly at P=0.05.
Each value is an average of three replicates.

A total of sixteen pure fungal colonies and twelve pure bacterial colonies were tentatively identified (Tables 1.3 and 1.4) as (AJF-: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 and16) for fungi and (AJB-: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11

and 12) for bacteria were re-isolated from mixed culture colonies. Fungal isolate AJF 15 was present only in one site with the least percentage frequency of 9% and isolates 2, 4, 8 and 13 were present in all the fields 100% frequency (Table 1.3). Bacterial isolate AJB 12 showed the least percentage frequency of 36% and isolates 1, 2, 4, 7 and 8 were present in all fields hence 100% frequency (Table 1.4)

Table-1.3. Percentage frequency of occurrence of fungal isolates in the rhizosphere soil samples.

Site/field Isolate (AJF)	1	2	3	4	5	6	7	8	9	10	11	sites present	% Frequency
1	+	+	+	+	+	+	-	+	+	-	-	08	73
2	+	+	+	+	+	+	+	+	+	+	+	11	100
3	+	+	+	+	+	+	+	+	+	-	-	09	82
4	+	+	+	+	+	+	+	+	+	+	+	11	100
5	+	+	+	+	+	+	+	+	+	+	-	10	91
6	+	+	+	+	+	+	+	+	+	+	-	10	91
7	+	+	+	+	+	+	+	+	+	+	-	10	91
8	+	+	+	+	+	+	+	+	+	+	+	11	100
9	+	+	+	+	+	+	+	+	+	+	-	10	91
10	+	+	-	+	+	+	+	-	-	-	-	06	55
11	+	+	+	+	+	+	+	+	+	+	+	11	100
12	+	+	-	-	+	+	+	-	+	+	+	08	73
13	+	+	+	+	+	+	+	+	+	+	+	11	100
14	-	-	+	-	-	+	-	-	+	-	-	03	27
15	-	-	+	-	-	-	-	-	-	-	-	01	09
16	-	-	+	+	-	+	-	+	-	-	-	04	36

Key: +. Present - Absent

Table-1.4. Percentage frequency of occurrence of bacterial isolates in the rhizosphere soil samples.

SITE/FIELD ISOLATE AJB	1	2	3	4	5	6	7	8	9	10	11	NO. of sites present	% Frequency
1	+	+	+	+	+	+	+	+	+	+	+	11	100
2	+	+	+	+	+	+	+	+	+	+	+	11	100
3	-	+	+	+	+	+	+	+	+	-	+	09	82
4	+	+	+	+	+	+	+	+	+	+	+	11	100
5	-	+	+	+	+	+	+	+	+	+	+	10	91
6	+	+	+	+	+	+	+	-	+	+	+	10	91
7	+	+	+	+	+	+	+	+	+	+	+	11	100
8	+	+	+	+	+	+	+	+	+	+	+	11	100
9	+	+	-	+	+	+	+	+	+	+	+	10	91
10	+	+	-	-	+	-	+	+	-	-	-	05	55
11	-	-	+	-	+	+	-	+	+	+	-	06	45
12	-	-	-	+	+	-	+	-	-	+	-	04	36

Key:+ Present - Absent

4.2. Morphological Identification of Fungi and Bacteria in the Rhizosphere of Sugarcane Variety CO 421.

4.2.1. Fungal Identification

Sixteen pure fungal isolates tentatively identified as AJF1- AJF 16 with varied morphological characteristics at day seven on PDA medium and the image of mycelia tip as observed under a phase contrast microscope magnification ×400 were described based on colony diameter, shape, margin, elevation, top and bottom colour, surface mycelia, hyphae and conidiophores shape (Tables 1.5 and 1.6).The morphologically described fungal isolates were identified in reference to Ellis, et al. [32]; Rajasankar and Ramalingam [41]; Alexopoulos, et al. [3]; Rocha, et al. [42] and Reynolds [43]; Williams [44]. Five of the isolates were identified to species level (AJF 4, 7, 8, 11 and 16) and seven isolates to genus level (AJF 1, 2, 3, 6, 10 and 13) and four isolates unidentified (AJF 9, 12, 41 and 15). *Trichoderma* was predominant with five isolates (AJF 3, 6, 7, 8 and 10) followed by *Aspergillus* four isolates (AJF 4, 11, 12 and 16) then *Rhizopus* (AJF 2), *Penicillium* (AJF 1) and *Alternaria* (AJF 13) one isolate each.

Table-1.5. Morphological characteristics of fungal colonies from the rhizosphere of sugarcane variety CO 421.

Isolate and plate	Diameter (mm)	Shape	Margin	Elevation	Color and surface		Hyphae	Conidia shape and conidiophores
					Top	Bottom		
Penicillium sp. AJF 1 (Plate 2)	10	Circular	Entire	Flat	Grayish green White bushy mycelia	Pale yellow	Septate	Globose/ Spherical
Rhizopus sp. AJF 2 (Plate 3)	60	Pyramid	Lobbed	Raised	Grayish black and powdery	Pale white	Aseptate	Globose
Trichoderma sp. AJF 3 (Plate4)	90	Circular	Entire	Flat	Green White mycelia	yellowish	Septate	Spherical
Aspergillus aureus. AJF 4 (Plate 5)	45	Circular	Lobbed	Raised	Yellow and brown at the centre Grooved	Purplish Red	Septate	Spherical
AJF 5 (Plate 6) unidentified	90	Circular	Entire	Raised	Yellow green Grooved and white mycelia	Yellow	septate	Spherical
Trichoderma sp. AJF 6 (Plate 7)	90	Circular	Entire	raised	Light green White mycelia	Yellow	septate	Spherical Green Branched conidiophores
Isolate and plate	**Diameter (mm)**	**Shape**	**Margin**	**Elevation**	**Color and surface**		**Hyphae**	**Conidia shape and conidiophores**
Trichorderma viride AJF 7 (Plate 8)	90	Circular	Entire	Flat	Dark green, yellowish at the centre White mycelia	Yellowish pale	Septate	Globose numerous and Green in colour
Trichoderma herzanium AJF 8 (Plate 9)	90	Circular	Entire	Raised	Dark green from the centre Many white mycelia	yellow	Septate	Ellipsoidal/ovalish Green in color
AJF 9 (Plate 10) Unidentified	90	Circular	Entire	Flat	White	cream	-	Spherical
Trichoderma sp. AJF 10 (Plate 11)	90	Circular	Entire	Raised	Green with grey centre	Pale yellow	Septate	Globose and green
Aspergillus niger AJF 11 (Plate 12)	40	Circular	Filamentous	Raised	Black with white margin Hairy	Yellowish	Septate	Spherical
Aspergillus AJF 12 (Plate 13)	5	circular	Lobbed	Raised	White Smooth	Cream	Aseptate	Spherical
Isolate and plate	**Diameter (mm)**	**Shape**	**Margin**	**Elevation**	**Color and surface**		**Hyphae**	**Conidia shape and conidiophores**
Alternaria sp. AJF 13 (Plate 14)	35	Circular	Filamentous	Raised	Grey with white towards the margin Hairy	Brown to black	Septate	Oval Club like
AJF 14 (Plate 15) Unidentified	63	Irregular	Lobbed	Raised	White hairy with grooves	Yellow	Aseptate	Spherical
AJF 15 (Plate 16) Unidentified	80	Irregular	Lobbed	Raised	White Rings curled	Cream	Aseptate	Spherical
Aspergillus flavus AJF 17 (Plate 18)	65	Circular	Filamentous	Raised	Yellow green White mycelia	Yellow brown	Septate	Spherical Conidia head

Table-1.6. Identity of Fungal isolates from rhizosphere of sugarcane variety CO 421.

Isolate	Kingdom	Phylum	Class	Order	Family	Genus	Species
AJF 1	Fungi	Ascomycota	Euascomycetes	Eurotiales	Trichomaceae	*Penicillium*	-
AJF 2	Fungi	zygomycota	zygomycetes	Mucorales	Mucoraceae	*Rhizopus*	-
AJF 3	Fungi	Ascomycota	Euascomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	-
AJF 4	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*A.aureus*
AJF5	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	-	
AJF 6	Fungi	Ascomycota	Euascomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	-
AJF 7	Fungi	Ascomycota	Euascomycetes	Hypocreales	Hypocreaceae	*Trichorderma*	*T.viride*
AJF 8	Fungi	Ascomycota	Euascomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	*T.herzanium*
AJF 9	Fungi	-	-	-	-	-	-
AJF 10	Fungi	Ascomycota	Euascomycetes	Hypocreales	Hypocreaceae	*Trichoderma*	-
AJF 11	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Aspergillus*	*A.niger*
AJF 12	Fungi	-	-	-	-	*Aspergillus*	-
AJF 13	Fungi	Ascomycota	Euascomycetes	Pleosporales	Pleosporaceae	*Alternaria*	-
AJF 14	Fungi	-	-	-	-	-	-
AJF 15	Fungi	-	-	-	-	-	-
AJF 16	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichomaceae	*Aspergillus*	*A.flavus*

Key: + Present - Absent

Table-1.7. Morphological characteristics of bacterial colonies and cells from rhizosphere of sugarcane variety CO 421

Isolate and plate	Colony shape	Elevation	Margin	Surface	Opacity	Color	Cell shape	Cell arrangement	Gram reactivity
AJB1 (Plate 18)	Circular	Raised	Entire	Glistening	Translucent	Cream	Comma	Single	Negative
AJB2 (Plate 19)	Circular	Flat	Entire	Glistening	Opaque	Cream	Short rods	Single	Negative
AJB3 (Plate 20) *Bacillus sp.*	Irregular	Flat	Entire	Glistening	Translucent	Cream	Rods	Chain	positive
AJB4 (Plate 21) *pseudomonas sp.*	Filamentous	Raised	Filiform	Glistening	Translucent	Cream	Short rods	Chain	Negative
AJB5 (Plate 22) *Pseudomonas sp.*	Circular	Raised	Entire	Glistening	Opaque	Cream	Rods	Single	Negative
AJB6 (Plate 23) *Pseudomonas sp.*	Circular	Raised	Entire	Glistening	Transparent	Cream	Short rods	Single	Negative
AJB7 (Plate 24) *Azobacter sp.*	Circular	Flat	Irregular	Glistening	Opaque	Cream	Short rods	Single	Negative
Isolate and plate	**Colony shape**	**Elevation**	**Margin**	**Surface**	**Opacity**	**Color**	**Cell shape**	**Cell arrangement**	**Gram reactivity**
AJB8 (Plate 25)	Circular	Flat	Irregular	Glistening	Opaque	White	Short rods	Double	Negative
AJB9 (Plate 26)	Circular	Flat	Irregular	Glistening	Translucent	White	Circular	Bunches	Negative
AJB10(Plate 27)	Circular	Flat	Entire	Glistening	Opaque	Yellow	Circular	Bunches	Negative
AJB11(Plate 28)	Circular	Flat	Wavy	Dull/dry	Translucent	Cream	Long rods	Single	Negative
AJB12(Plate 29) *Bacillus sp.*	Circular	Raised	Wavy	Glistening	Translucent	Cream	Short rods	Double	Positive

4.2.2. Bacterial Identification

Twelve pure bacterial isolates were tentatively identified as AJB1 – AJB 12 with varied colony and cell morphological characteristics on NA medium. The isolates were morphologically described based on colony shape,

elevation, margin, surface, opacity and colour followed by Cell shape, arrangement and gram reactivity as observed under a phase contrast microscope magnification×1000 (Table 1.7). All the isolates were gram negative except isolate AJB 3 and AJB12. The morphologically described isolates were identified in reference to Bergey's manual of determinative bacteriology identification flow chart. Six of the isolates identified to genus level (AJB 3, 4, 5, 6, 7 and 12) and six isolates unidentified (AJB 1, 2, 8, 9, 10 and 11). *Pseudomonas* was predominant with three isolates (AJB 4, 5 and 6) followed by *Bacillus* two isolates (AJB 3 and 12) and *Azobacter* (AJB 7) one isolate.

Table-1.8. Identity of Bacterial isolates from rhizosphere of sugarcane variety CO 421

Code name	Kingdom	Phylum	Class	Order	Family	Genus
AJB 1	Monera	-	-	-	-	-
AJB 2	Monera	-	-	-	-	-
AJB 3	Monera	Firmicutis	Bacilli	Bacillales	Bacillaceae	*Bacillus*
AJB 4	Monera	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	*pseudomonas*
AJB 5	Monera	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
AJB 6	Monera	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
AJB 7	Monera	Proteobacteria	Gamma proteobacteria	Pseudomonadales	Azobacteraceae	*Azobacter*
AJB 8	Monera	-	-	-	-	-
AJB 9	Monera	-	-	-	-	-
AJB 10	Monera	-	-	-	-	-
AJB 11	Monera	Proteobacteria	-	-	-	-
AJB 12	Monera	Firmicutis	Bacilli	Bacillales	Bacillaceae	*Bacillus*

5. DISCUSSION

5.1. Population of Fungi and Bacteria in the Rhizosphere of Sugarcane Variety CO 421.

The findings from this study indicate that both fungi and bacteria were present in the rhizosphere of sugarcane in agreement with what has been reported by many researchers [18]; [34] confirming its ability to host numerous and diverse microbes than bulk soil.

The population of the microflora was higher in the rhizosphere than non rhizosphere in all the locations, in agreement with previous studies by Kelechi and Chiaka [26]; Tailor and Joshi [45] and Afzal, et al. [2]; Deshmukh, et al. [16] on rhizosphere of sugarcane varieties CO 86032 and CO 0265. The disparity in the rhizosphere and bulk soil microbial population could be due to sugarcane plant roots releasing exudates containing different organic and inorganic compounds that stimulated development of active microbial population in the soil [3]. The nature and concentration of these organic constituents and the corresponding ability of the microorganisms to utilize them as sources of energy may contribute to the disparity in population of the two regions [14].

Bacterial population was more than that of fungi in all the fields which is in agreement to the findings by Cappuccino and Sherma [11]; Tamilarasi, et al. [21] where bacteria recorded higher population of 2.8×10^{6} cfu/g of soil than fungi 1.0×10^{4}cfu/g of rhizosphere soil on selected medicinal plants and the findings of Athul, et al. [9]; Tamilarasi, et al. [21] on rhizosphere soils of vanilla crop that recorded 4.1×10^{5} cfu/g for bacteria to 3.45×10^{3} cfu/g for fungi. These numbers were lower than the population in this study (bacteria: 1.265×10^{7} cfu/g and fungi: 4.89×10^{4} cfu/g) of sugarcane rhizosphere soil, probably due to disparity in soil type, plant species, root type and microbial interactions. Similar results have been reported by Athul, et al. [9]; Bello and Utang [10] .The high bacteria population may be attributed to greater rhizosphere effect on bacteria than fungi.

The significant differences in microbial population between the ten fields from which rhizosphere soil was obtained in this study could have been due to variations in sugarcane plant age in the fields and pH of the soils. Microbial activity increases with plant age and declines towards maturity probably due to the plants secreting exudates in reduced quality and quantity that may contain antimicrobial metabolites [29].

The present study isolated 28 pure isolates (16 fungi and 12 bacteria). The dominant fungal genera in all the fields were *Trichoderma*, *Rhizopus*, *Aspergillus* and *Alternaria* and bacteria were *Bacillus*, *Pseudomonas* and *Azobacter* . This is in agreement with the findings of other scientists [15]; [46]. High sporulation in *Rhizopus*, *Aspergillus* and *Alternaria may* have contributed to their dominance. *Aspergillus* is known to produce toxins that may prevent growth of other fungal species [13].

5.2. Specific Fungi and Bacteria in the Rhizosphere of Sugarcane Variety CO 421.

Morphology of single cells or colony characteristics remains a reliable parameter for bacterial and fungal species identification and still has a significant taxonomic value [31]. The isolates exhibited diverse morphological characteristics based on macroscopic characteristics such as colony diameter, colony surface and reverse colour, colony shape and microscopic features including cell shape, gram reactivity, conidia shape, conidia colour and nature of hyphae this is in accordance to a study by Afzal, et al. [2]; Pisa, et al. [39]. In the present study, *Aspergillus*, *Penicillium*, *Trichoderma*, *Rhizopus* and *Alternaria* genera of fungi in the rhizosphere of CO 421 sugarcane variety were identified. The findings are also similar to those reported by Deshmukh, et al. [16]; Makut and Owolewa [29]. Variations in microbes may be attributed to differences in environmental variables such as pH and temperature. Makut and Owolewa [29]; Kumar, et al. [30] isolated and identified using morphological features *Aspergillus*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium* and *Rhizopus* from rice rhizosphere soils in India similar to the microbes isolated in sugarcane rhizosphere probably because they belong to the same family Poaceae. Different plant species host specific microbial communities [5] and that diversity and composition of the microbial taxa in the rhizosphere can be affected by plant species [11].

This study identified *Bacillus*, *Pseudomonas* and *Azobacter* genera of bacteria in the rhizosphere of sugarcane, in agreement to the findings of Ashraf, et al. [8]; Tailor and Joshi [45]. Sule and Oyeyiola [34]; Rajasankar and Ramalingam [41] identified similar genera of bacteria in sugarcane rhizosphere except for the genera *Azomonas and Mesorhizobium* that were not identified in this study, probably due to variation in environmental factors. Prashar, et al. [40]; Williams [44] working with pearl millet rhizosphere, identified Streptomyces, *Pseudomonas*, *Flavobacterium*, *Bacillus*, *Streptococcus* and *Staphylococcus* genera of bacteria, four of which were not identified in this study ,may be because of this was a different plant species.

5.3. Conclusions

This study has confirmed that there is high population of fungi and bacteria in the rhizosphere soil samples of CO 421 sugarcane variety crop. Rhizosphere has a stimulatory effect on the population of the micro flora making the population higher in the rhizosphere than in the non rhizosphere soil. Bacteria were more stimulated than fungi hence had a greater number of colonies and population per gram of soil. *Aspergillus*, *Penicillium*, *Trichoderma*, *Rhizopus* and *Alternaria* genera of Fungi and *Bacillus*, *Pseudomonas* and *Azobacter* genera of Bacteria were found in the rhizosphere of CO 421 sugarcane variety plants. *Trichoderma* and *Pseudomonas* genera were predominant. The results of this study demonstrate the diversity of the sugarcane rhizosphere microbial community and have a broader implication for improving the ability to manipulate them for improved sugarcane growth and health. The existence of high population of rhizosphere microorganisms in sugarcane variety CO 421 plants is an indication that there are diverse exudates produced by the roots of sugarcane, therefore this finding provides us with the opportunity to optimize the biological functions of the plant soil ecosystem, which can lead to increased benefits of sugarcane production. Further research should be done on the efficacy of the microbial biocontrol agents in sugarcane varieties.

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REFERENCES

[1] M. Abdulkadir and S. Waliyu, "Screening and isolation of the soil bacteria for ability to produce antibiotics," *European Journal of Applied Sciences*, vol. 4, pp. 211-215, 2012. *View at Google Scholar*

[2] H. Afzal, A. Haq, S. Shazad, and S. Qamar, "Morphological identification of aspergillus species from soils of Thata district Pakistan," *International Journal of Agriculture and Applied Sciences*, vol. 5, pp. 105-117, 2013.

[3] C. J. Alexopoulos, C. W. Mims, and M. Blackwell, *Introductory mycology*, 4th ed. India: Wiley, 2002.

[4] E.-A. Al-Nur and M. A. Abdulmoneim, "Contribution to the knowledge of soil fungi in Sudan rhizosphere mycoflora of sugarcane at Kenana sugar estate," *International Journal of Botany*, vol. 3, pp. 97-102, 2007. *View at Google Scholar | View at Publisher*

[5] A. H. Alwathnani, K. Perveen, R. Tahmaz, and S. Alhaqbani, "Evaluation of biological control potential of locally isolated antagonist fungi against Fusarium oxysporum under in vitro and pot conditions," *African Journal of Microbiology Research*, vol. 6, pp. 312-319, 2012. *View at Google Scholar*

[6] R. L. Berendsen, M. J. C. Pieterse, and A. H. M. Bakker, "The rhizosphere micro biome and plant health," *Trends in Plant Science*, vol. 17, pp. 478-486, 2012. *View at Google Scholar*

[7] A. J. M. Angel, O. S. Reena, S. Aysha, N. P. Valli, and P. Vinothkumar, "Isolation of siderophore producing bacteria from rhizosphere soil and their antagonistic activity against selected fungal plant pathogens," *International Journal of Current Microbiology and Applied Sciences*, vol. 2, pp. 59-65, 2013. *View at Google Scholar*

[8] A. A. Ashraf, M. Rasool, and M. S. Mirza, "Nitrogen fixation and Indole acetic acid production potential of bacteria isolated from rhizosphere of sugarcane (Saccharum Officinarum L.)," *Advances in Biological Research*, vol. 5, pp. 348-355, 2011. *View at Google Scholar*

[9] K. S. R. Athul, A. Aju, and M. S. Jisha, "Biocontrol of fusarium wilt of vanilla (Vanilla Planifolia) using combined inoculation of trichoderma sp. and pseudomonas sp," *International Journal of Pharma and Bio Sciences*, vol. 3, pp. 706 - 716, 2012. *View at Google Scholar*

[10] O. S. Bello and G. A. Utang, "An ecological survey of microorganisms associated with plantain roots (rhizosphere)," *American Journal of Agriculture and Biological Sciences*, vol. 6, pp. 567-570, 2011. *View at Google Scholar | View at Publisher*

[11] G. J. Cappuccino and N. Sherma, *Microbiology: A laboratory manual*, 8th ed. San Francisco: Pearson Education, 2008.

[12] H. Chanda, G. Nivedita, T. Pravin, and D. Kondiram, "Influence of sugarcane monocropping on rhizosphere microflora, soil enzymes and NPK status," *International Journal of Pharma and Bio Sciences*, vol. 2, pp. 188-202, 2011.

[13] M. A. Chandrashekar, P. K. Soumya, and N. S. Raju, "Fungal diversity of rhizosphere soils in different agricultural fields of Nanjangud Taluk of Mysore district, Karnataka, India," *International Journal of Current Microbiology and Applied Sciences*, vol. 3, pp. 559-566, 2014. *View at Google Scholar*

[14] S. P. G. Gaddeya, B. P. Niharika, and R. P. K. Kumar, "Isolation and identification of soil mycoflora in different crop fields at Salur Mandal," *Advanced Applied Sciences Research*, vol. 3, pp. 2020-2026, 2012. *View at Google Scholar*

[15] N. R. Damle and S. W. Kulkarni, "Enzymatic potential of bacteria isolated from the rhizosphere of Aegle marmelos (Bael Tree)," *World Journal of Environmental Biosciences*, vol. 1, pp. 86-89, 2012.

[16] R. B. Deshmukh, S. S. Dange, P. V. Jadhav, S. S. Deokule, and N. A. Patil, "Studies on the mycoflora in the rhizosphere of sugarcane (Saccharum officinarum L.)," *International Journal of Bioassays*, vol. 2, pp. 674-676, 2013. *View at Google Scholar*

[17] S. Dua and S. S. Sidhu, "Effectiveness of rhizosphere bacteria for control of root rot disease and improving plant growth of wheat (Triticum Aestivum L.)," *Journal of Microbiology Research*, vol. 2, pp. 26-35, 2012. *View at Google Scholar | View at Publisher*

[18] A. Sood, S. Sharma, V. Kumar, and R. L. Thakur, "Antagonism of dominant bacteria in tea rhizosphere of Indian Himalayan region," *Journal of Applied Science sand Environmental Management*, vol. 11, pp. 63-66, 2007. *View at Google Scholar*

[19] Food and Agriculture Organization of the United Nations FAO, "Micro propagation for quality seed production in sugarcane in Asia and the pacific." Retrieved from www.appari.org, 2008.

[20] D. B. Nekade, "Bacterial diversity in sugarcane (Saccharum Officinarum L.) rhizosphere of saline soil," *International Research Journal of Biological Sciences*, vol. 2, pp. 60-64, 2013. *View at Google Scholar*

[21] S. Tamilarasi, K. Nanthakumar, K. Karthikeyan, and Lakshamanaperumalsami, "Diversity of root associate microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of Coriandum savitum," *Journal of Environmental Biology*, vol. 29, pp. 127- 134, 2008.

[22] A. Gowsalya, V. Ponnusami, and K. R. Sugumaran, "Isolation of bacteria from soil sample for exo- polysaccharide production," *International Journal of ChemTech Research*, vol. 6, pp. 2925-2928, 2014. *View at Google Scholar*

[23] S. Ibrahim and M. A. Ramha, "Isolation and identification of fungi associated with date fruits sold at Bayero University, Kano Nigeria," *Journal of Pure and Applied Sciences*, vol. 2, pp. 127-130, 2009. *View at Google Scholar | View at Publisher*

[24] J. Jamoza, C. Kirungu, R. Amolo, P. Wanyama, G. Riungu, and J. Wafubwa, *Sugarcane growers guide*, 3rd ed.: Kenya Sugar Research Foundation, 2013.

[25] S. Kalaiselviselvaraj and A. Panneerselvam, "Invitro assessment of antagonistic activity of trichoderma sp. against sarocladium oryzae causing seath rot in paddy," *International Journal of Applied Biology and Pharmaceutical Technology*, vol. 2, pp. 179-183, 2011.

[26] M. O. Kelechi and M. A. Chiaka, "The rhizosphere effect on the bacterial Genera associated with crude oil polluted soil ecosystem," *Current Research in Microbiology and Biotechnology*, vol. 2, pp. 495-500, 2014.

[27] C. Kimberly and B. Elsa, "Identification of bacterial species," *Tested Studies for Laboratory Teaching*, vol. 24, pp. 103-130, 2003. *View at Google Scholar*

[28] Z. Kumalawati, Y. Musa, N. Amin, L. Asrul, and I. Ridwan, "Exploration of arbuscular mycorrhizal fungi from sugarcane rhizosphere in South Sulawesi," *International Journal of Scientific & Technology Research*, vol. 3, pp. 201-203, 2015.

[29] M. D. Makut and O. A. Owolewa, "Antibiotic producing fungi present in the soil environment of keffi metropolis, Nasarawa state of Nigeria," *Trakia Journal of Sciences*, vol. 9, pp. 33-39, 2011.

[30] P. K. R. Kumar, G. Hermanth, P. S. Niharika, and S. K. Kolli, "Isolation and identification of soil mycoflora in agricultural fields at Tekkali Mandal in Srikakulam district," *International Journal of Advances in Pharmacy, Biology and Chemistry*, vol. 4, pp. 484-490, 2015. *View at Google Scholar*

[31] N. P. Shiny, G. Gaddeyya, and K. P. K. Ratna, "An Investigation on Soil mycoflora of different crop fields at narasannapeta Mandal, Srikakulam District," *International Research Journal of Environment Sciences*, vol. 2, pp. 38-44, 2013. *View at Google Scholar*

[32] D. Ellis, S. Davis, H. Alexious, R. Handke, and R. Bartley, "Description of medical fungi." 2nd Edn., Retrieved from http:// www.mycology.adelaide.edu.av, 2007.

[33] B. Nandhini and M. R. Josephine, "Study of bacterial and fungal diversity in potted soil," *International Journal of Current Microbiology and Applied Sciences*, vol. 2, pp. 1-5, 2013.

[34] I. O. Sule and G. P. Oyeyiola, "Fungi in the Rhizosphere and rhizoplane of cassava cultivar TME 419," vol. 4, pp. 18 - 30, 2012.

[35] P. Nannipieri, J. Ascher, M. T. Ceccherini, L. Landi, G. Pietramellara, G. Renella, and F. Valori, "Microbial diversity and microbial activity in the rhizosphere," *Ciencia del. Suelo (ARGENTINA)*, vol. 25, pp. 89-97, 2007. *View at Google Scholar*

[36] V. Nihorimbere, M. Ongena, M. Smargiassi, and P. Thonart, "Beneficial effect of the rhizosphere microbial community for plant growth and health," *Biotechnology Agrononomy Society and Environment*, vol. 15, pp. 327-337, 2011. *View at Google Scholar*

[37] H. S. Nzioki, J. E. Jamoza, C. O. Olweny, and J. K. Rono, "Characterization of physiologic races of sugarcane smut (Sporisorium Scitamineum) in Kenya," *African Journal of Microbiology Research*, vol. 4, pp. 1694-1697, 2010. *View at Google Scholar*

[38] J. Ong'ala, B. Mulianga, N. Wawire, G. Riungu, and D. Mwanga, "Determination of sugarcane smut prevalence in the Kenya sugar industry," *International Journal of Agriculture Innovations and Research*, vol. 4, pp. 153- 157, 2015. *View at Google Scholar*

[39] G. Pisa, G. S. Magnani, H. Weber, F. E. M. Souza, R. A. Monterio, and L. M. Cruz, "Diversity of 16S rRNA genes from bacteria of sugarcane rhizosphere soil," *Brazilian Journal of Medical and Biological Research*, vol. 44, pp. 1215-1221, 2011. *View at Google Scholar* | *View at Publisher*

[40] P. Prashar, N. Kapoor, and S. Sachdeva, "Structural and functional diversity of rhizobacteria of pearl millet in semi-arid agro climatic zone," *Asian Journal of Plant Science and Research*, vol. 2, pp. 599-606, 2012. *View at Google Scholar*

[41] R. Rajasankar and C. Ramalingam, "In vitro screening of saccharum officinarum L. rhizophere bacteria for plant growth promotion traits," *International Journal of Advanced Life Sciences*, vol. 1, pp. 40-49, 2012. *View at Google Scholar*

[42] U. N. Rocha, S. L. van Overbeek, and D. J. Elsas, "Exploration of hitherto-uncultured bacteria from the rhizosphere," *FEMS Microbiology Ecology*, vol. 69, pp. 313-328, 2009. *View at Google Scholar* | *View at Publisher*

[43] J. Reynolds, "Biochemical tests and unknown identification in: Biology 2420 lab manual," pp. 103-143, 2011.

[44] J. Williams, *Simplified fungi identification key* vol. 37: The University of Georgia Co- operative Extension Service, 2001.

[45] A. Tailor and H. B. Joshi, "Characterization and optimization of siderophore production from pseudomonas fluorescens strain isolated from sugarcane rhizosphere," *Journal of Environmental Research and Development*, vol. 6, pp. 688-694, 2012. *View at Google Scholar*

[46] P. Tshikudo, R. Nnzeru, K. Ntushelo, and F. Mudau, "Bacterial species identification getting easier," *African Journal of Biotechnology*, vol. 12, pp. 5975-5982, 2013. *View at Google Scholar* | *View at Publisher*