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Different cover promote sandy soil suppressiveness to root rot disease of cassava caused by *Fusarium solani*

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Sandy soils of 19 arable fields were analyzed for disease suppressiveness against root rot disease of cassava, caused by *Fusarium solani* CFF109. Analysis of chemical, physical, biochemical, microbial population and activity, and soil characteristics were assessed. Soils with different management histories and covers most commonly found in the region were collected to build a greenhouse experiment, to evaluate the natural suppressiveness of soils against cassava root rot. The severity was submitted with all data across correlation and multivariate analyses to find correlation between disease suppressiveness and abiotic or biotic soils attributes. Differences in disease suppressiveness were found between the treatments for cassava root rot. Significant disease suppression was found in 37% of the soils. The sandy soil covered with consortium of *Zea mays* + *Vigna unguiculata* was the most efficient in suppressiveness against disease caused by *F. solani.* The data indicated significant correlation between soil characteristics and suppressiveness cassava root rot. The soil supressiveness against cassava root rot was favored by increase by total bacteria, fluorescent group bacteria populations and activity of acid and alkaline phosphatase and β-glucosidase.

Key words: Disease suppression, soil characteristics, enzyme activity, soil communities, abiotic factor, biotic factor.

INTRODUCTION

Manihot esculenta Crantz, popularly known as 'cassava' is the most widely cultivated species of the genus *Manihot* (Nassar et al., 2008). It is a perennial shrub cultivated in many continents as an important food source, because cassava roots are high in calories and is one of the largest producers of carbohydrates (Adeoti, 2010), it is the fourth most consumed source of carbohydrates in the tropics, and have high starch making it the staple food for over 500 million people (Tonukari, 2004).

Cassava has been inserted as raw material in various industrial processes (Buensanteai and Athinuwat, 2012) and is considered one of the main crops for ethanol production in Thailand (Chaisinboon and Chontanawat, 2011). The higher demand for cassava has made producing countries to increase their areas of planting (Adeoti, 2010). However, the continuous and intensive management

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practices, with few attentions from producers with regard to sanitary and physiological quality of cuttings used (Fukuda, 1991), occurrence of diseases, among others, this crop has shown low levels of productivity.

The root rot has been responsible for major losses in cassava production. In Brazil, the second largest producer in the world, the fungi that cause root rot of cassava are mainly *Phytophthora* spp. and *Fusarium solani* which account for 30 and 70% losses, respectively (Fukuda, 1991).

F. solani can survive in plant debris or in the soil through resistance structures as chlamydospores for a long time (Hasanzade et al., 2008), or in the form of spores or mycelium infected or dead tissue. As control is difficult and handling with chemicals is expensive and inefficient, besides causing environmental pollution with pesticides, the use of resistant varieties and cultural practices are important strategies in management of this disease. Another way is sustainability by induction of suppressiveness of the soil. This has properties that limit the development of the disease (Diab et al., 2003). Several studies have demonstrated the power of soils to suppress diseases caused by a diversity pathogens including *Fusarium oxysporum*, *Pythium* sp., *Rhizoctonia solani, Streptomyces saran* and *Scytalidium lignicola* (Mazzola and Gu, 2002; Silva et al., 2013).

The objective of this study was to characterize aspects related to suppressiveness or conductivity of soils with different management histories for targeting programs of integrated control of root rot of cassava caused by *F. solani*.

MATERIALS AND METHODS

Soil samples

Arable fields of 19 different crop systems in tropical dry sandy soil of semiarid were sampled in dry season. These soils are from Pernambuco state, Brazil with different history of management (Table 1). The region's climate, is the CSA type "temperate Mediterranean" mesothermal with a hot dry summer, according to Köppen classification. The areas present similar climate, topography and altitude. The average annual rainfall is around 750 to 1250 mm. The predominant soils are Regolithic Neosols. The annual average temperature is between 15 and 18°C.

From each field five independent soil samples of about 20 kg were taken at 0-10 cm depth with 10 m distance between each sampling. Physical and chemical parameters were analyzed. Part of the soil collected was immediately cooled to 4°C for microbiological and biochemical analyzes.

Soil suppressiveness

The isolate identified as *F. solani* was obtained from material collected on farms producing cassava in Jupi city of Pernambuco- Brazil (8° 42′ 23″ S, 36° 25′ 3″ O). Pathogenicity tests was conducted and culture deposited at the Coleção de Culturas de Fungos Fitopatogênicos da UAG/Universidade Federal Rural de Pernambuco (UFRPE), Brazil with the code CFF109.

The inoculum of pathogen was prepared by addition of 8 mm discs containing fungal structure of *F. solani* CFF109 with 21 days of growth. Such disks were added to flasks containing 250 g of rice peeled and 150 mL of distilled water and sterilized. Subsequently, the inoculum was dried, crushed and weighed at a rate of 0.5 g added to the soil. The number of colony forming units (CFU) was estimated by Johnson and Curl (1972) and quantified in 1000 CFU in 5 g of inoculum.

Soil samples were placed in plastic pots (4 kg capacity) and infested with *F. solani* CFF109 by deposition of 5 g colonized substrate, followed by homogenizing the mixture. After 14 days, two cuttings of "Branquinha" cassava were planted in pots. The cuttings measured 8 to 10 cm were previously disinfected with sodium hypochlorite 3% dried in a protected environment.

After 90 days the plants were analyzed by three evaluators and were done by finding external symptoms such as yellowing and wilting and then isolating the stem and roots present for evaluation of internal symptoms evidenced by the dark coloration in vascular tissue of the plant. Disease severity was measured by a scale and disease index was calculated according to McKiney (1923), using the assignment of notes to the symptoms presented. Plants 0 = no symptoms, plants 1 = with less than 10% to 25% injuries, plants 2 = 25 to 50%, plants 3 = with 50 to 75%, plants 4 = 75 to 100% (dead plants). The experimental design was completely randomized design with 19 treatments, with four replications.

Chemical and physical attributes

Chemical attributes were determined: pH in water (1:2.5), available P, exchangeable K, Na, Al, Ca and Mg and the total organic carbon (TOC) according to Yeomans and Bremner (1988). The P, Na and K were extracted by Mehlich I, while Na and K were determined by flame photometry.

Physical attributes were texture (proportions of sand, silt and clay) by the hydrometer method, soil bulk density (SBD) and particle (PD) and total porosity (TP), which was obtained by the equation (EN = 1 - (Ds / Dp)). The retained moisture at field capacity (FC) and permanent wilting point (PWP) were determined with the extractor Richards, at pressures of -0.01 and -1.5 MPa, respectively. The values of FC and PWP were used for calculated available water (AW) in the soil for crops:

AW = FC – PWP.

Microbial and biochemical attributes

The total fungi (TF), total bacteria (TB), endospore-forming bacteria (BFE) and fluorescent group bacteria (FGB) population were obtained by serial dilutions as Johnson and Curl (1972).

In the determination of microbial biomass carbon (MBC), the samples were subjected to irradiation process. The extraction of the biomass was performed using as extractant K_2SO_4 0.5 M. To each 20 g of soil was added 80 mL K_2SO_4 0.5 M. The carbon in the extracts was determined by colorimetric method (Bartlett and Ross, 1988).

The soil basal respiration (SBR) was determined by quantifying the carbon dioxide (CO_2) released in the process of microbial respiration (CO_2 evolution) by the method of adsorption alkaline, with the moisture of the soil samples adjusted to 60% capacity field (Anderson and Domsch, 1985).

The metabolic quotient (qCO_2) was calculated as the ratio between the SBR and MBC expressed in micrograms of C-CO_2 per microgram per day of MBC. The microbial quotient (qMIC) was

Table 1. Field and use history of soils used to evaluate the natural suppressiveness of sandy soils against cassava root rot caused by *F. solani.*

Code	History	Textural class
TDF	This area was used for comparative. The native vegetation consisting of small trees, mostly Jurema (*Mimosa tenuiflora*) which lose their leaves seasonally, characteristic of Brazilian tropical dry forest.	Sandy
CAS	Monoculture (*Manihot esculenta)*, preparation of cultivation area with plowing and harrowing with addition of cow manure before planting.	Sandy
GRA	Monoculture of elephant grass (*Pennisetum purpureum*) for more than five years.	Sandy loam
PAST	Monoculture of pangola grass (*Digitaria decumbens*).	Sandy loam
BEA	Monoculture of bean (*Phaseolus vulgaris*) under a traditional cultivation system, fertilized with cow manure at the time of preparation, without irrigation management.	Sandy
PIG	Monoculture of pigeon pea (*Cajanus cajan*), traditionally managed system showing accumulation of organic material at the base of the plants.	Sandy
BA	Area where the traditional management of burning was conducted about 10 days before sample collection.	Sandy
CORCOW	Intercropping cultivation (*Zea mays* + *Vigna unguiculata*).	Sandy loam
CASPC	Intercropping cultivation (*Manihot esculenta* + *Cajanus cajan* + *Vigna unguiculata*). under traditional management carried out with periodic weeding.	Sandy loam
COT	Monoculture of cotton (*Gossypium hirsutum*), located next to the highway with little weeding.	Sandy loam
ORA	Monoculture of orange (*Citrus sinensis*), the orchard features an accumulation of plant material on the soil surface.	Sandy loam
FEN	Monoculture of fennel (*Pimpinella anisum*) on a slope with many stones.	Sandy loam
PAS	Monoculture of passion fruit (*Passiflora edulis* f. *flavicarpa*) under traditional management with drip irrigation in stony soil hard to excavate.	Sandy loam
CASH	Monoculture of cashew nuts (*Anacardium occidentale*), trees aged between 10 and 15 years.	Sandy loam
CAB	Monoculture of cabbage (*Brassica oleracea*) under a traditional cultivation system accomplished with addition of chemical fertilizers and inputs for pest and disease control.	Sandy loam
PRI	Monoculture of prickly-pear cactus (*Opuntia cochenillifera*) without recent management and with the presence of weeds between the lines of the planting system.	Sandy loam
PEP	Monoculture of sweet pepper (*Capsicum annuum*). under sprinkler irrigation and where spraying for pest and disease control was performed two days before sample collection.	Sandy loam
CUK	Monoculture of cucumber (*Cucumis sativus*), under sprinkler irrigation.	Sandy loam
EUC	Monoculture of eucalyptus (*Eucalyptus globulus*) with little accumulation of plant material on the soil surface.	Sandy loam

TDF = *Mimosa tenuiflora*; CAS = *Manihot esculenta*; GRA = *Pennisetum purpureum*; PAST = *Digitaria decumbens*; BEA = *Phaseolus vulgaris*; PIG = *Cajanus cajan*; BA = burned area; CORCOW = *Zea mays* + *Vigna unguiculata*; CASPC = *Manihot esculenta* + *Cajanus cajan* + *Vigna unguiculata;* COT = *Gossypium hirsutum;* ORA = *Citrus sinensis;* FEN = *Pimpinella anisum;* PAS = *Passiflora edulis;* CASH = *Anacardium occidentale;* CAB = *Brassica oleracea;* PRI = *Opuntia cochenillifera;* PEP = *Capsicum annuum;* CUK = *Cucumis sativus;* EUC = *Eucalyptus globulus.*

calculated by MBC/TOC, according to Sparling (1992).

The soil enzymatic activities involved in C, P, S and N cycles was determined. The β-glucosidase (BGLU) (3.2.1.21) was determined according to the methodology proposed by Eivazi and Tabatabai (1988), using ρ-nitrophenyl-β-D-glucoside (SIGMA ALDRICH) as substrate. The acid and alkaline phosphatase (ALK PHOSPH/ AC PHOSPH), (EC 3.1.3) were determined by Eivazi and Tabatabai (1977); aryl-sulfatase (ARY) (EC 3.1.6.1) by Tabatabai and Bremmer (1972) and Urease (URE) (EC 3.5.1.5) by Kandeler and Gerber (1988).

Statistical analysis

The data of severity were subjected to analysis of variance (ANOVA) and compared by the Scott-Knott test at $p \leq 0.05$ used to determine whether differences in suppressiveness among different soils were statistically significant. Subsequently, we selected the most suppressive soils and we submitted all the biotic and abiotic soil factors to multivariate analysis of principal components analyses (PCA) to determine which key variables is responsible for detecting differences between treatments. Finally, to compare the attributes involved in the soil suppressiveness against cassava root rot, the severity were compared with chemical, physical, microbial and biochemical data by linear correlation of Pearson, at 5% probability.

RESULTS AND DISCUSSION

Among the sandy soil under 19 different cover, 42% were

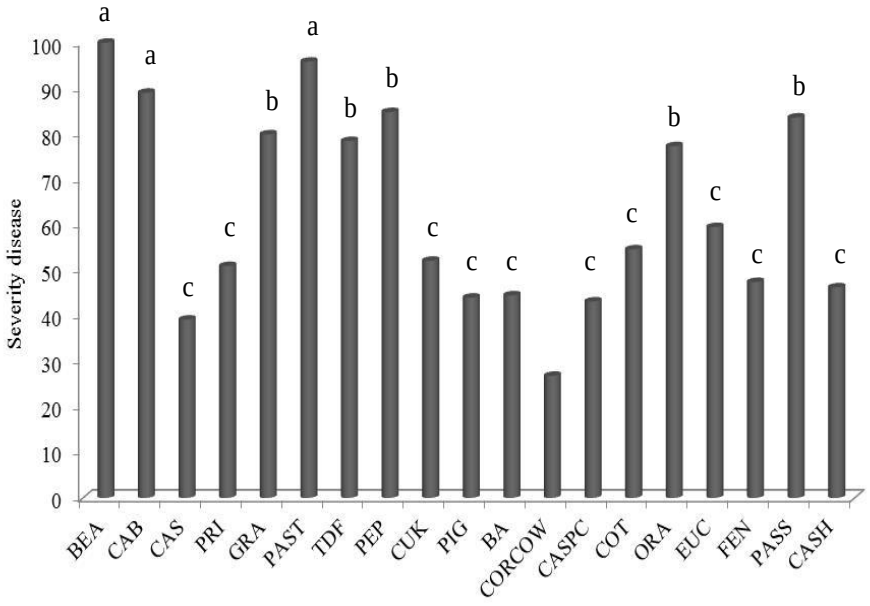

Figure 1. Disease severity of natural soil supressiveness to cassava rot root, caused by *F. solani*. TDF = *Mimosa tenuiflora*; CAS = *Manihot esculenta*; GRA = *Pennisetum purpureum*; PAST = *Digitaria decumbens*; BEA = *Phaseolus vulgaris*; PIG = *Cajanus cajan*; BA = burned area; CORCOW = *Zea mays* + *Vigna unguiculata*; CASPC = *Manihot esculenta* + *Cajanus cajan* + *Vigna unguiculata;* COT = *Gossypium hirsutum;* ORA = *Citrus sinensis;* FEN = *Pimpinella anisum;* PAS = *Passiflora edulis;* CASH = *Anacardium occidentale;* CAB = *Brassica oleracea;* PRI = *Opuntia cochenillifera;* PEP = *Capsicum annuum;* CUK = *Cucumis sativus;* EUC = *Eucalyptus globulus*. * Means followed by the same letter did not differ by Scott-Knott test at (p≤0.05).

considered conducive, 21% moderately conducive and 37% considered suppressive. There were significant differences (p ≤0.05) between the severity of root rot of cassava in soil with different historical management (Figure 1). The highest disease suppression was found for system of consortium CORCOW (*Zea mays* + *Vigna unguiculata*) with medians of 26% of severity of the disease. This occurred probably due the diversity of plants that attracts a greater diversity of microorganisms that may be involved in soil suppressiveness (Friberg et al., 2005). A study on natural suppressiveness of 10 organic farms with arable fields soil against *R. solani* AG2.2IIIB in sugar beet showed high correlation between suppressiveness, bacterial diversity and fungal activity (Postma et al., 2008).

The diversity and quantity increase of microorganisms acting in soil suppressiveness against cassava root rot demonstrates a poor competitive ability of the causal agent of this disease, *F. solani* CFF109. However, the poor competitive ability of other *Fusarium* species has been reported, although there is a study that reports the opposite (Nyvall and Kommedahl, 1973). Therefore, the suppressive ability of soil to *Fusarium* species may vary according to aggressiveness and/or competitive ability of the isolates present in that soil (Knudsen et al., 1999). Furthermore, the microbial communities of rhizosphere from different plants may vary not only in number but also the structure that directly affects the capacity of soils suppressive to diseases caused by *Fusarium* (Abadie et al., 1998).

The interaction between hosts and pathogens (Termorshuizen et al., 2006; Ghini et al., 2007) can differ in the power of soil suppressiveness, due to the specificity and ecology of the causal agent in soil. For example, the soil inhabitant pathogen *R. solani* has a saprogenic stage and can grow in organic matter while *Verticillium* need a host to survive (Postma et al., 2008).

The second best treatment with suppressive capacity of cassava root rot was the CAS soil. Several studies show advantages in introducing organic materials to increase the power of soils suppressive against various pathogens such as *R. solani*, *Verticillium dahliae*, *Phytophthora nicotianae*, *Phytophthora cinnamomi, Fusarium oxysporum* f. sp. Lini and *Scytalidium lignicola* (Diab et al., 2003; Termorshuizen et al., 2006; Silva et al., 2013). The application of organic additives are beneficial to induce the production of fungi toxic compounds such as organic acids and ammonia (Termorshuize et al., 2006), these materials function as substrates for microorganisms that exert antagonistic competition with pathogens (De

Clercq et al., 2004).

The other treatments considered suppressive were in the order of most to least suppressive: CORCOW > CAS > CASPC>PIG>BA>CASH>FEN, all with severity ranging from 26 to 47% (Figure 1). Such treatments were used for multivariate analysis of principal component analyze.

The variation among suppressiveness soils to cassava root rot based on biotic and abiotic parameters is illustrated by PCA. The variation explained by the first two axes was 65.46% for the analysis based on abiotic (Figure 2A) and 55.51% for the biotic soil attributes (Figure 2B). Abiotic parameters that best describe the variation in soil supressiveness to root rot of cassava, caused by *F. solani* were: TP> Mg> WA> PWP> PD> Na> Al> FC> SBD. These parameters explain 39.68% of the variation (Axe 1; Figure 2A). The biotic soil parameters that best described the variation were: ARY> TF> qMic> SBR>EFB=FGB> TB> BGLU> MBC (Axe 1; Figure 2B). Cumulatively, these attributes explain 34.02% of the variation of soils suppressiveness.

The grouping of suppressive soils to cassava root rot differ between biotic and abiotic variables, therefore, the multivariate CPA was performed separately (Figure 2A for abiotic factors and Figure 2B for abiotic factors). The fact that biotic and abiotic attributes cannot group similar soils has been reported in other studies (Termorshuizen et al., 2006), corroborating the present study.

The correlation coefficient between disease severity and soil abiotic and biotic parameters are shown in Table 2. Among the abiotic factors, the increase of field capacity (FC) was accompanied by increased disease severity (positive correlation) suggesting that soils with higher field capacity has lower power suppressive to disease. In contrast, soils with higher amounts of Na have greater powers to suppress cassava root rot, caused by *F. solani*. In a study evaluating the ability of two soils suppressive: Low humic soil (LA) and Haplic soil (HA) club root disease of Chinese cabbage caused by *Plasmodiophora brassicae*, it was observed that the soil with a lower Na content, haplic soil (HA) showed less suppressive capacity, with a higher disease index (Murakami et al., 2000), corroborating the positive effect of Na in the soil suppressiveness.

We observed significant correlations between the severity of cassava root rot and biotic soil parameters such as: TB, FGB, ALPH, ACPH and BGLU, all with negative correlations with severity. Studies have reported that the presence of certain bacteria act directly on the disease suppressiveness, including *Pseudomonas* spp. (Mazzola and Gu, 2002), *Streptomyces* spp. (Tuitert et al., 1998) and *Lysobacter* spp. (Postma et al., 2008), suggesting some mechanisms of bacteria antagonism against plant pathogenic fungi. In addition to the bacterial populations, total microbial activities, metabolic structure of soil and microbial communities have been associated with suppressiveness diseases (Tuitert et al., 1998), regardless

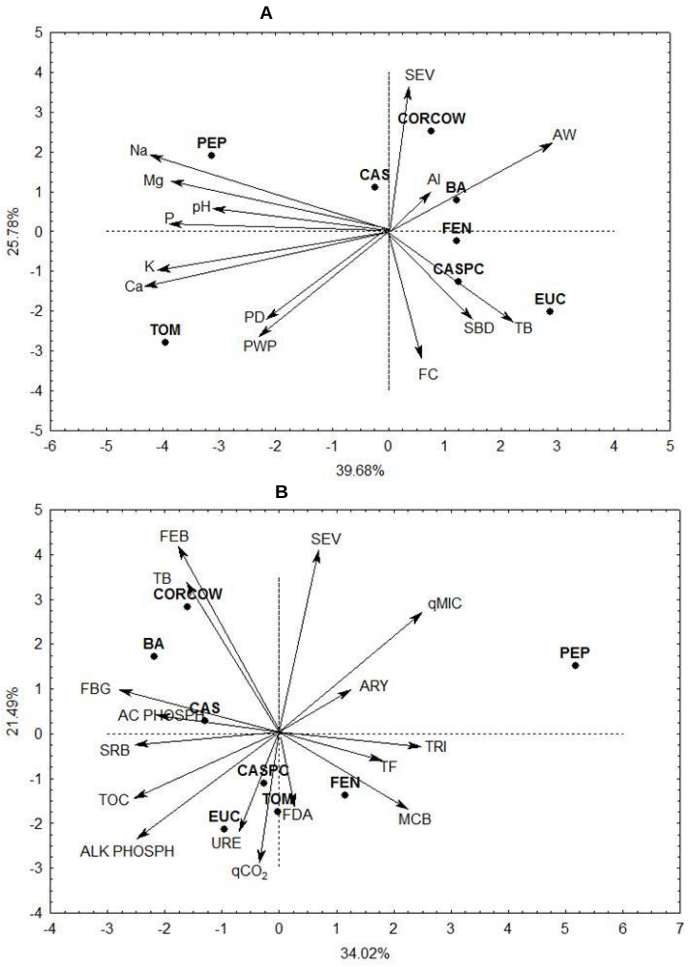

Figure 2. Principal component analysis (PCA) based on disease suppressiveness of sandy soil of different management against cassava root rot disease. (A) Abiotic soil parameters; (B) biotic soil parameters. SBD = soil bulk density, PD = particle density, TP = total porosity, FC = field capacity, PWP = permanent wilting point, AW = available water, TF = total fungi, TB = total bacteria, EFB = endospore-forming bacteria, FGB = fluorescent group bacteria, MBC = microbial biomass carbon, SBR = soil basal respiration, *q*CO2 = metabolic quotient, Qmic =microbial quotient, BGLU = β-glucosidase, ACPH = acid phosphatase, ALPH = alkaline phosphatase, ARY = aryl-sulfatase, URE = urease. CAS = *Manihot esculenta*; PIG = *Cajanus cajan*; BA = burned area; CORCOW = *Zea mays* + *Vigna unguiculata*; CASPC = *Manihot esculenta* + *Cajanus cajan* + *Vigna unguiculata;* FEN = *Pimpinella anisum;* CASH = *Anacardium occidentale.*

of soil type (Murakami et al., 2000) and by several factors (Janvier et al., 2007), mainly soil biotic attributes.

We found a significant correlation between cassava root rot suppression in natural sandy soils and the activity of three enzymes: ALPH, ACPH and BGLU that should be used as a new mode of evaluation of cassava root rot suppression. Many experiments aimed at disease suppressiveness are performed with highly commercial plants, due to the importance of cassava to small farmers, they

Table 2. Correlation coefficient between severity of cassava root rot against abiotic and biotic parameters of sandy soil from different history of management.

Abiotic	Severity
Particle density	-0.37
Soil bulk density	-0.22
Total porosity	-0.08
Field capacity	0.56*
Permanent wilting point	0.06
Available water	0.40
pH	0.00
P	-0.13
K	0.32
Ca	0.09
Mg	-0.18
Na	-0.52*
Al	-0.01
Biotic	
Total fungi	-0.41
Total bacteria	-0.52*
Endospore-forming bacteria	-0.16
Fluorescent group bacteria	-0.50*
Total organic carbon	0.39
Microbial biomass carbon	0.34
Soil basal respiration	0.09
Microbial quotient	0.00
Metabolic quotient	0.11
Acid phosphatase	-0.57*
Alkaline phosphatase	-0.52*
β-glucosidase	-0.58*
Aryl-sulfatase	-0.15
Urease	0.34

*Significant correlation coefficient by Pearson correlation analysis. The level of 5% probability.

should sought for an alternative disease management that is very important for all producing countries. Therefore, we recommended increasing the diversity of microorganisms in soil by use of a consortium and application of organic matter in the soil.

Conclusions

There is a potential for natural suppressiveness of sandy soils in suppressing cassava root rot caused by *F. solani* CFF109. The analysis of the total population of bacteria, fluorescent group bacteria, acid phosphatase, alkaline phosphatase and β-glucosidase enzyme activities are biotic attributes that could serve as a tool to evaluate the soil suppressiveness. We concluded that sandy soils with consortium system and with organic matter have the power to suppress cassava root rot.

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