Chapter

Sisal Bole Rot: An Important but Neglected Disease

Valter Cruz-Magalhães, Jackeline Pereira Andrade, Yasmim Freitas Figueiredo, Phellippe Arthur Santos Marbach and Jorge Teodoro de Souza

Abstract

Sisal (*Agave sisalana*) is one of the main sources of hard natural fibre and raw materials for the industry, medicine and handicrafts. Sisal yields a coarse and strong fibre that is increasingly being used in composite materials for automobiles, furniture, construction and plastic and paper products. Extracts of sisal contain substances with anti-inflammatory, antimicrobial and anthelmintic activities. Sisal is adapted to warm environments with low rainfall and is an excellent option for cultivation in semiarid conditions, where other crops cannot be grown. The world's largest sisal producers are Brazil, Tanzania, China, Kenya and Madagascar. Sisal is a labour-intensive crop with great socio-economical importance as it is cultivated in poor areas employing familiar labour. Sisal bole rot is the main disease of sisal, responsible for substantial losses in producing countries. The disease is caused by certain species of the genus *Aspergillus*, especially the ones belonging in the section *Nigri*. The main symptoms are yellowing of the aerial parts and the red-coloured rot of the bole, which causes the plant to die. In this review we are going to address the taxonomy of the causal agents, disease diagnosis and epidemiology and disease management, with emphasis on biological control.

Keywords: *Aspergillus welwitschiae*, *Agave sisalana*, biological control, disease management, semiarid regions

1. Introduction

Agave sisalana Perr. ex. Engelm is a monocotyledonous, xerophytic, succulent plant that belongs in the *Asparagaceae* family. The genus *Agave* has more than 200 species, and Mexico is their centre of origin and dispersion, where they have high economic importance and several industrial applications [1, 2]. This genus is able to grow in different conditions, as well as to show excellent adaptation to environments with warm climate, high luminosity and prolonged droughts [3, 4]. Tolerance to abiotic stresses is a striking feature of *A. sisalana*, which confers good performances to this species under conditions that limit the development of most plants [4]. This tolerance is related to morphological and physiological characteristics, such as the CAM metabolism (crassulacean acid). This type of metabolism allows for greater efficiency in water use, higher carbon uptake during the night and low nutritional demand when compared to C3 and C4 plants [1, 5–7].

Sisal is a monocarpic plant, and the emission of an inflorescence characterises the end of its vegetative cycle, which can occur between 8 and 30 years. The plant multiplies vegetatively through bulbils produced on the inflorescence pole or by stolons that emerge from the rhizome (subterraneous stem) of adult plants. The use of bulbils is the most common form of propagation, but stolons can also be used. The production of seeds is rare, and induction techniques are necessary when this is the objective [8–10]. Most species of *Agave* are highly endemic and have high levels of genetic variation within populations and low differentiation between populations [11]. This limited diversity hinders the establishment of germplasm banks and the search for genes that confer desirable characteristics to these plants.

Agave sisalana is a good producer of hard natural fibres [1]. The fibre extracted from this plant occupies the sixth position of importance and represents 2% of the world production of plant fibres [12]. This product is extracted from the leaves of the plant and is traditionally used in the manufacture of cords and ropes [9]. In addition, it is widely used in various industrial sectors. Amongst several applications, sisal fibre has been increasingly used in the reinforcement of building materials, furniture, panels and automobile upholstery [1, 12, 13]. In addition to the various applications and industrial uses, sisal fibre has advantages over synthetic fibres for having lower density (lighter) and lower production cost and is biodegradable and recyclable. Therefore, the use of sisal fibre fits in the growing world tendency that favours the use of sustainable natural resources with less environmental impact [14, 15].

There has been a growing interest in the use of waste or by-products from *Agave* species in biotechnological processes [16, 17]. After fibre extraction the residue is usually discarded [18]. This residue accounts for 98% of the total biomass of the plant and has potential to be used as raw material for biofuels, especially because it is not directly used as food [6, 12, 19]. In order to exploit the economic value of this material, a joint initiative between the Common Fund for Commodities, the United Nations Industrial Development Organization (UNIDO) and the Tanzanian sisal industry financed the first commercial plant for the production of biogas [12]. In addition to some medicinal properties reported [20, 21], *A. sisalana* also produces compounds that have different biological properties [18] of great interest in the pharmaceutical industry such as hecogenin [12, 21–23]. All of the above features place sisal as a strategic species to be exploited in tropical semiarid regions and in temperate latitudes with drought resulting from global climate change [16, 19, 24].

The main world producers of sisal fibre are Brazil, Tanzania, China, Kenya and Madagascar [25]. Other countries, such as Mexico, South Africa, Mozambique, Angola, Indonesia, Thailand, Haiti and Cuba, also produce but in smaller quantities. According to FAO reports, in 2011 Brazil alone produced more than 111 thousand tons of sisal fibre [12].

In Brazil, the semiarid region of Bahia province (northeastern Brazil) is responsible for more than 95% of the country's sisal production [26]. Other provinces that produce smaller amounts of sisal in Brazil are Paraiba, Rio Grande do Norte and Ceará [27]. It is estimated that more than 150,000 families are directly linked to the producing chain of this crop, totalling more than 700,000 small farmers, and more than half a million direct and indirect jobs are involved in activities related to the maintenance, harvesting, extraction and processing of fibre [28–30]. In this sense, sisal has an important economic and social role of the semiarid region of Brazil.

Sisal management is simple because this plant exhibits tolerance to various abiotic stresses. Even under minimal management conditions, the plant presents good development and consequently good fibre production, with low nutritional requirements [12]. However, although it presents all these adaptive advantages to stress conditions, the main problem is of phytosanitary origin. Sisal bole rot, the main disease of sisal, has caused considerable damage to the crop [31]. This disease

causes the death of infected plants, and despite the economic and social importance of sisal, there are few government efforts to control the disease.

In this chapter we introduce the sisal bole rot disease, a neglected disease that represents the main challenge for sisal production in Brazil and other countries of the world. In addition, we discuss some aspects involved in its symptomatology, aetiology, epidemiology and management. The majority of the results that will be shown were obtained in Brazil, where most of the research on sisal bole rot was done.

2. Bole rot disease: symptoms and epidemiology

The disease was first reported in production areas of Tanzania and Brazil [31, 32]. In Brazil, since the 1990s, the commercial production of sisal has been

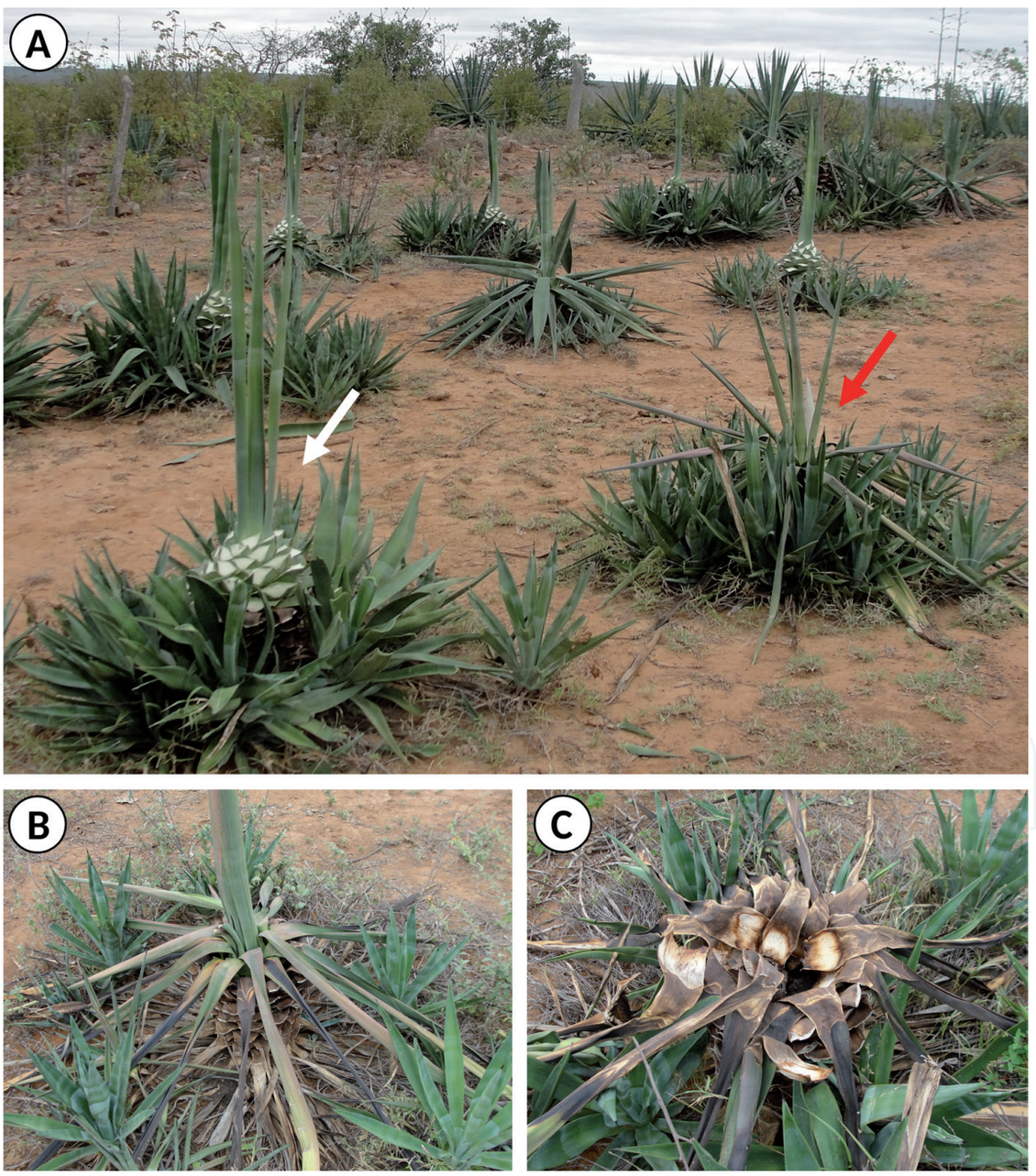

Figure 1.
Adult sisal plants under field conditions. (A) Healthy adult plant (white arrow) after leaf harvest for fibre extraction next to an adult plant showing the external symptoms of sisal bole rot (red arrow). The diseased plant has wilted and yellowish leaves that cannot be used for fibre extraction and therefore was not harvested. (B) and (C) Plants killed by the pathogen.

declining due to economical crises and the occurrence of this disease [33]. Diseased plants produce leaves that are not suitable for fibre extraction as they lose their turgescence, and although these diseased plants survive for some time, they die with the progress of the disease (**Figure 1**) [35]. Plants at advanced stages of the disease are easily identified by the symptoms, which include wilting and yellowing of the aerial part (**Figure 1A**). The main internal symptom of the disease is rotting of the stem with reddening of the tissues, a response of the plant to fungal colonisation. It is thought that there is no relationship between the phenological stage of the plant and the establishment of the disease, since the fungus is capable of infecting both plantlets (**Figure 2E**) and adult plants (**Figures 1** and **2**).

It was reported that the pathogen depends on mechanical injuries and natural openings, mainly on physiologically stressed plants, to start the infection process [32]. In this sense, it is possible that wounds made by insects or by tools used in crop management, such as harvest of the leaves and cultural practices, are ways of pathogen penetration [32, 35, 36]. The histopathology of diseased plants showed that the pathogen penetrates the tissues of the host from the outside, that is, from the epidermis to the parenchyma and later to the central cylinder of the plant [37].

Abreu [36] studied the spatiotemporal distribution of sisal bole rot in producing areas of Bahia Province, Brazil, and found that the disease was present in all

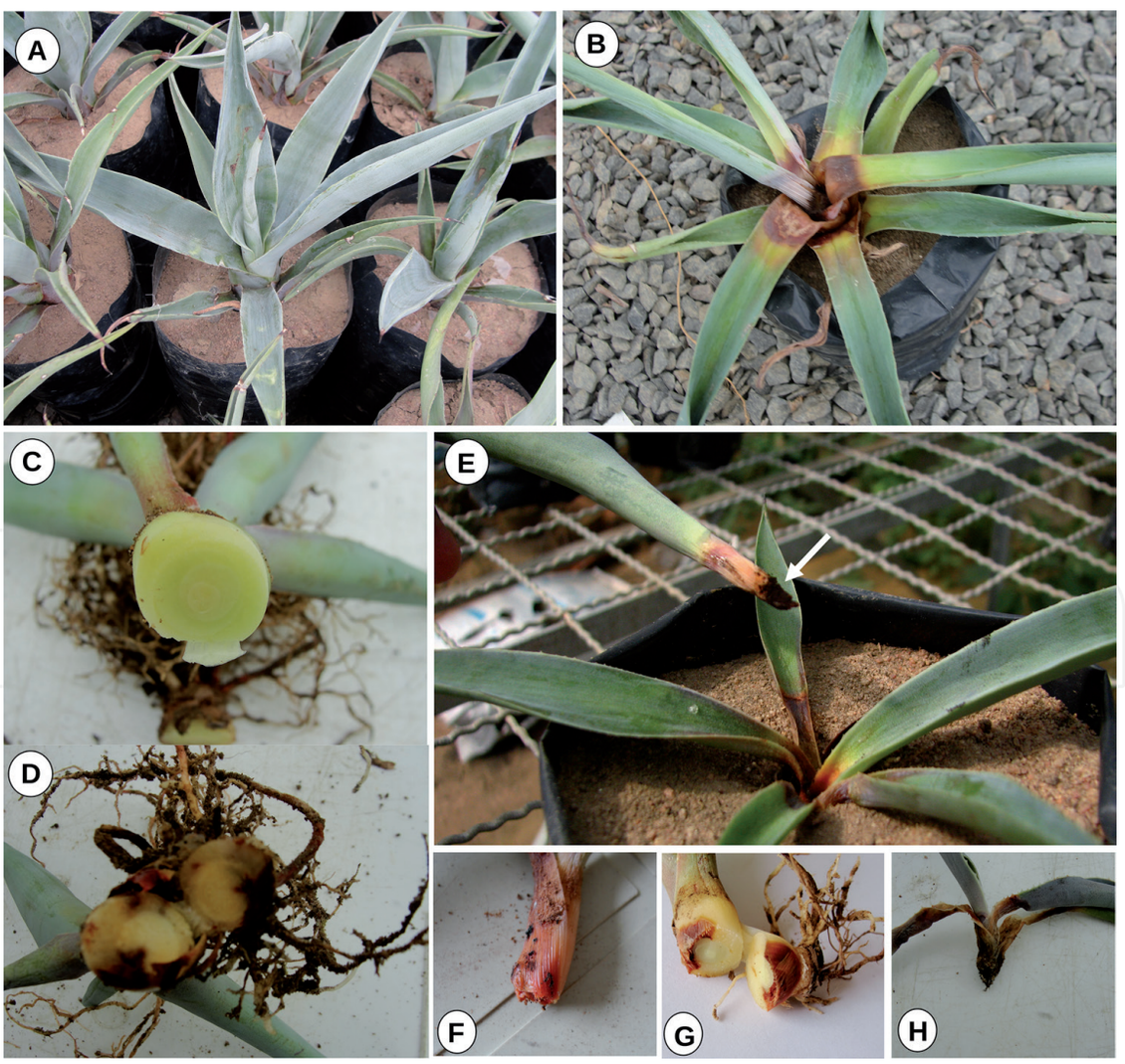

Figure 2.
Sisal plantlets with symptoms of sisal bole rot under greenhouse conditions. (A) Healthy sisal plantlets and (B) diseased plantlet with symptoms of sisal bole rot. (C) Stem of healthy plant. (D, F and G) Intermediate symptoms of sisal bole rot, characterised by rotting of the stem. (E and H) Dead plants. The white arrow indicates the production of conidia after colonisation of plant tissues.

the studied farms (prevalence of 100%) and, on average, 35% of the plants were infected by the pathogen. This study also showed that the distribution of the disease occurs randomly in the cultivated areas [36]. In the case of sisal bole rot, incidence evaluations are more important than severity, as there are no measures that slow down the progress of the disease.

The lack of more studies on epidemiological aspects of sisal bole rot in different areas where the disease occurs directly impacts the establishment of phytosanitary management practices. More information on these aspects could contribute to the development of strategies to reduce the incidence of the disease. For the moment, what is known is that preventive measures should be employed to avoid the establishment of the pathogen in the area.

3. Causal agents

The disease was first observed in areas of sisal production in Tanzania in the 1930s but was only reported in the 1950s [32]. The causal agent was isolated from diseased plant parts and identified as *Aspergillus niger*. In this study, the authors reported fruiting bodies of *A. niger* in exposed plant tissues and also pointed out that the occurrence of the disease was linked to environmental conditions and the nutritional status of the plant [32]. The first report of this disease in Brazil also occurred in the 1950s, when Machado [38] described a rot of the base of sisal

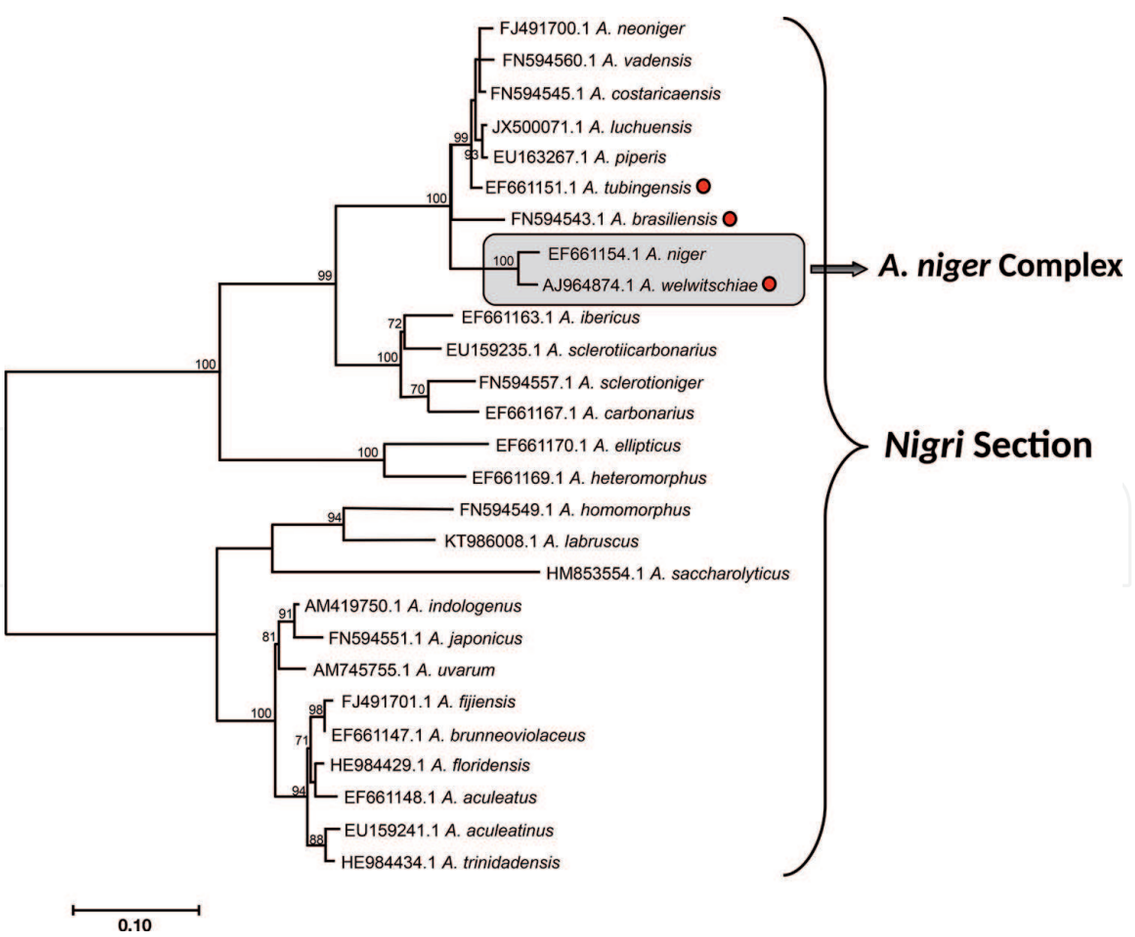

Figure 3.
Phylogenetic tree of the 27 valid species belonging in the Nigri section of Aspergillus. The red circles indicate species shown to cause sisal bole rot in the A. niger complex. The tree was constructed with sequences of the calmodulin gene, with 456 nucleotides aligned using the maximum likelihood (ML) method and the K2 + G + I substitution model. The bootstrap analysis was performed with 1000 resamplings. The scale represents the number of substitutions per site.

stem in the province of Paraíba, Brazil [39]. In Bahia, the largest sisal-producing province in Brazil, the disease was first noticed in a commercial plantation by researchers from the Agency for Agricultural Development of Bahia (EBDA) and Embrapa Semiárido (Brazilian Agricultural Research Institute) in the municipality of Santaluz [33].

In Tanzania and in Brazil, the disease was initially associated with the species *A. niger*. The aetiology of the disease was determined by Koch's postulates from tissue fragments of diseased sisal plants [40]. Species of the genus *Aspergillus* are filamentous fungi belonging in the phylum *Ascomycota* [41]. *Aspergillus niger* and other closely related species form a cluster of morphologically similar species, collectively known as the section *Nigri* (**Figure 3**). The *Nigri* section is comprised of 27 valid species that contain the *A. niger* complex (**Figure 3**). All these species have as main characteristic the formation of black-coloured conidia, uniseriate or biseriate conidiophores and dark colonies (**Figure 4**) [42]. The taxonomy of the section *Nigri* is very complex because many species of this group are difficult to distinguish morphologically [41]. The morphological criteria were the only ones used to identify these species for a long time, and for this reason, many species were misidentified [43, 44].

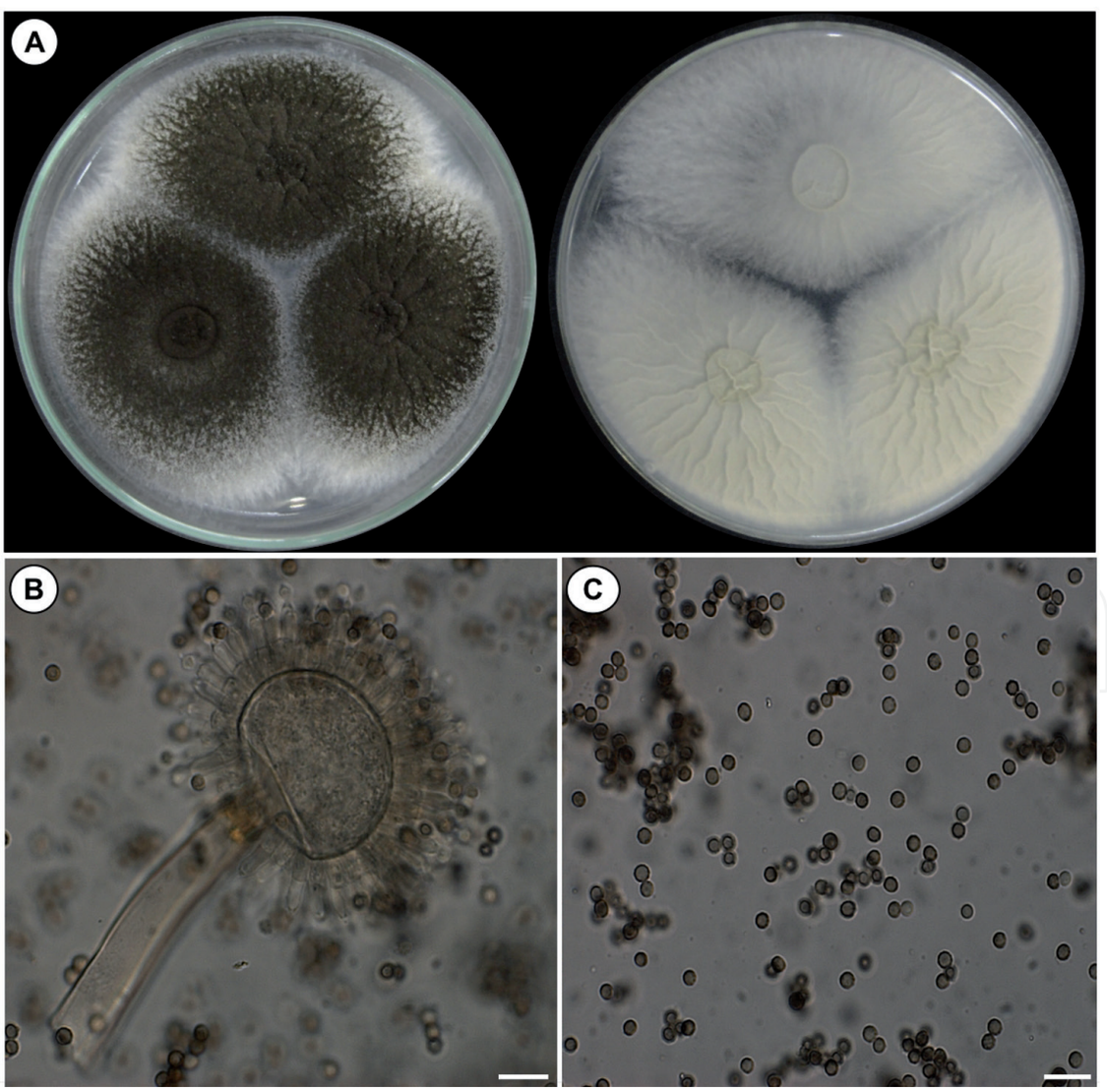

Figure 4.
Macro- and micromorphology of Aspergillus welwitschiae isolated from diseased sisal plants. (A) Obverse and reverse of a plate containing mycelial growth of colony on Blakeslee's malt extract (MEAbl), growing at 25°C for 7 days. (B) Conidiophores of A. welwitschiae and (C) conidia. Scale bars =10 μm.

The polyphasic taxonomy integrates molecular, physiological, metabolite production and morphological data for the identification and description of new species of the section *Nigri* [45–48]. The regions recommended for the identification and description of species in the genus *Aspergillus* are fragments of the ITS region of the ribosomal DNA, calmodulin (*caM*), beta-tubulin (*benA*) and the beta subunit of the RNA polymerase (*rpb2*). However, caM sequences were proposed as the most informative markers for the section *Nigri* [49]. The gene *benA* is very informative for the uniseriate/aculeatus clade; however, care must be taken not to use the wrong set of primers (Bt2a/Bt2b) that can also amplify *tubC*, a paralog of *benA*, resulting in misidentification. The alternative primer pair ben2f/Bt2b should be used instead [50]. The other methods used in the polyphasic approach include growth on different media and temperatures, production of secondary metabolites and measurement of all fungal structures [44, 45].

The initial studies implicated only *A. niger* as the cause of bole rot disease because the authors only took the morphological features of the pathogen into account [32, 40]. Further studies including sequences of the ITS region of the ribosomal DNA and a fragment of the transcription and elongation factor of the RNA polymerase (tef1-alpha) also identified *A. brasiliensis* and *A. tubingensis* in addition to *A. niger* as agents of the disease [31]. Recently, Duarte et al. [37] identified molecular phylogeny strains of *Aspergillus* sp. of the section *Nigri* obtained from diseased plants using a fragment of the calmodulin gene and proposed that *A. welwitschiae* and not *A. niger* is the causal agent of sisal bole rot disease. However, these authors did not include *A. niger* in their study, and therefore, further investigations are still needed to evaluate the ability of other species in the section *Nigri* to cause the disease, including *A. niger*.

4. Disease management

There are no effective control methods available for bole rot disease [51]. Mechanical lesions are used by the pathogen as penetration sites, and this has direct implications for crop management since leaf harvest causes wounds in the plant [32, 36]. Additionally, the pathogen may be spread through the use of tools contaminated in diseased plants.

Most farmers use plantlets from stolons to establish new plantations, and infected plant material contributes to the spread of the disease to new areas. Therefore, the establishment of new areas using healthy plant material is thought to be one of the most effective ways to prevent the introduction of the pathogen. Removal and destruction of diseased plants from the plantations, balanced fertilisation to prevent stresses and disinfestation of the tools used in diseased plants are other measures recommended to decrease the incidence and avoid the spread of the disease to new areas [52].

Another method investigated to manage the disease is the use of antagonistic microorganisms [53, 34]. Chemical control was never investigated probably because the causal agents are soilborne fungi and farmers have little financial resources. Biological control is an environmentally friendly and viable method to control plant pathogens [54, 45]. Antagonistic bacteria were shown to have potential to control the bole rot disease [53, 34]. Several strains of an undescribed species of *Burkholderia* and strains of *Bacillus* decreased the incidence and severity of the disease under field conditions (**Figures 5** and **6**) [53, 34]. Therefore, it is possible to establish programmes aimed at the development of biological products to manage the disease in the field.

Figure 5.
Management of sisal hole rot disease with antagonistic bacteria. (A) and (B) Plantlets treated with Burkholderia sp. and inoculated with the pathogen A. welwitschiae in the field. (C) and (D) Sisal plants inoculated with A. welwitschiae only under field conditions (positive control).

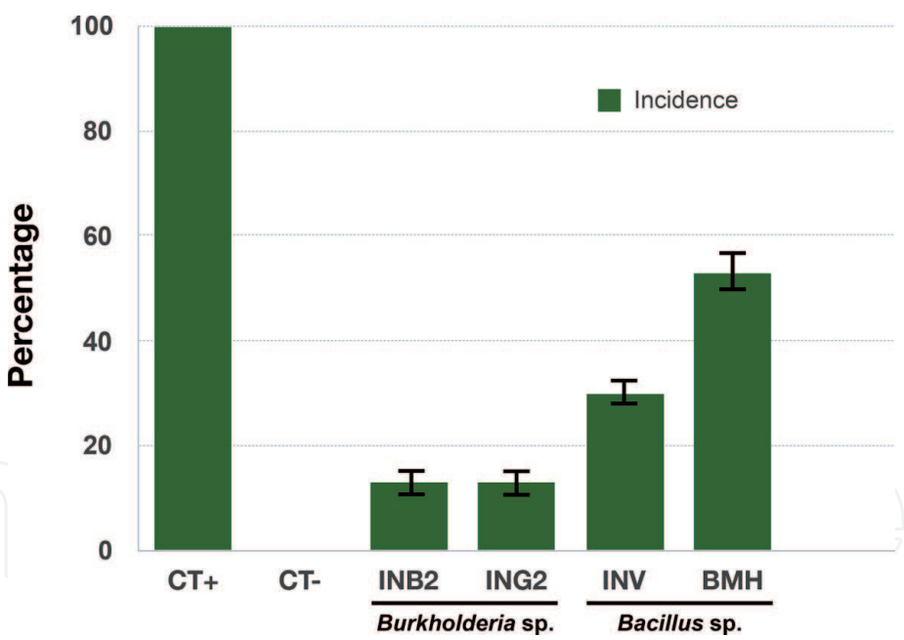

Figure 6.
Incidence of sisal hole rot disease by the application of Burkholderia and Bacillus strains under field conditions. The means represent 25 replicates per treatment. The negative control was treated with water only (CT-) and positive control with A. welwitschiae (CT+). Error bars represent the standard error of the means.

5. Outlook

Little is known about the mechanisms used by the pathogen to infect the plant, although *Aspergillus* shows a typical necrotrophic behaviour [37]. More information on the pathogenicity mechanisms could be obtained by the use of omics tools, such as RNAseq, to identify genes expressed by the pathogen during infection. Other microorganisms can influence the establishment and progress of the disease, and in this sense it will be interesting to study the comparative microbiome of diseased

and healthy plants. This information may be used to engineer the microbiome to keep the plants healthy, as it has been attempted for other agricultural crops [55].

Sisal bole rot cannot be controlled by any single method, and therefore, the integration of control measures must be adopted. Resistant cultivars are not available for this crop, and unfortunately there are no breeding programmes focusing on sisal bole rot [9]. Breeding programmes are limited by the low genetic diversity of natural populations out of Mexico.

Preventive measures are thought to be the most effective ways to control bole rot, and these include (i) the use of healthy planting material, (ii) balanced fertilisation to avoid nutritional stresses and (iii) maintaining adequate soil humidity levels to avoid physiological imbalances [52]. When these measures are not able to contain the pathogen, removal of diseased plants is recommended to decrease the source of inoculum of the pathogen [52]. One challenge in this regard is the development strategies to identify diseased plants before the dispersal of pathogen propagules.

Sisal residues are commonly used to fertilise plants in the field [52], but only the fermented residue is suitable for this purpose as fresh residues stimulate the spread of the pathogen [56]. Information such as these could be disseminated to farmers to contribute to the management of the disease. Sisal farmers in many parts of the world do not have access to information on the technical aspects of sisal, depend on familiar labour and have little financial resources to invest in the crop. The information generated so far on the management of the disease through the use of antagonistic bacteria are promising, but it is still necessary to develop it into products that can be used by the farmers. New studies aiming at formulating and distributing biological products should be encouraged to contribute to the sustainability of this crop in the long run. The general lack of research on bole rot classifies it as a neglected disease that deserves more attention from research institutes and the government.

Acknowledgements

The authors thank the Brazilian agencies CNPq and CAPES for the financial support.

Author details

Valter Cruz-Magalhães[1], Jackeline Pereira Andrade[2], Yasmim Freitas Figueiredo[1], Phellippe Arthur Santos Marbach[3] and Jorge Teodoro de Souza[1*]

1 Federal University of Lavras, Lavras, MG, Brazil

2 Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brazil

3 Reconcavo da Bahia Federal University, BA, Brazil

*Address all correspondence to: jorge.souza@dfp.ufla.br

References

[1] Nava-Cruz NY, Medina-Morales MA, Martinez JL, Rodriguez R, Aguilar CN. Agave biotechnology: An overview. Critical Reviews in Biotechnology. 2015, 2015;**35**:546-559. DOI: 10.3109/07388551.2014.923813

[2] Trejo-Torres JC, Gann GD, Christenhusz MJ. The Yucatan Peninsula is the place of origin of sisal (*Agave sisalana*, *Asparagaceae*): Historical accounts, phytogeography and current populations. Botanical Sciences. 2018;**96**:366-379. DOI: 10.17129/botsci.1928

[3] Pinos-Rodríguez JM, Zamudio M, González SS, Mendoza GD, Bárcena R, Ortega ME, et al. Effects of maturity and ensiling of *Agave salmiana* on nutritional quality for lambs. Animal Feed Science and Technology. 2009;**152**:298-306. DOI: 10.1016/j.anifeedsci.2009.05.002

[4] Sarwar MB, Ahmad Z, Rashid B, Hassan S, Gregersen PL, Leyva MDLO, et al. De novo assembly of *Agave sisalana* transcriptome in response to drought stress provides insight into the tolerance mechanisms. Scientific Reports. 2019;**9**:1-14. DOI: 10.1038/s41598-018-35891-6

[5] Kant P. Could Agave be the species of choice for climate change mitigation? In: Working Paper IGREC-11. New Delhi: Institute of Green Economy, IGREC; 2010

[6] Escamilla-Treviño LL. Potential of plants from the genus *Agave* as bioenergy crops. Bio Energy Research. 2012;**5**:1-9. DOI: 10.1007/s12155-011-9159-x

[7] Gross SM, Martin JA, Simpson J, Abraham-Juarez MJ, Wang Z, Visel A. De novo transcriptome assembly of drought tolerant CAM plants, *Agave deserti* and *Agave tequilana*. BMC Genomics. 2013;**14**:563. DOI: 10.1186/1471-2164-14-563

[8] Das T. Micropropagation of *Agave sisalana*. Plant Cell, Tissue and Organ Culture. 1992;**31**:253-255. DOI: 10.1007/BF00036233

[9] Nikam TD. High frequency shoot regeneration in *Agave sisalana*. Plant Cell, Tissue and Organ Culture. 1997;**51**(3):225-228. DOI: 10.1023/A:1005976304198

[10] Nikam TD, Bansude GM, Kumar KA. Somatic embryogenesis in sisal (*Agave sisalana* Perr. ex. Engelm). Plant Cell Reports. 2003;**22**(3):188-194. DOI: 10.1007/s00299-003-0675-9

[11] Eguiarte LE, Aguirre-Planter E, Aguirre X, Colín R, González A, Rocha M, et al. From isozymes to genomics: Population genetics and conservation of Agave in México. The Botanical Review. 2013;**79**(4):483-506. DOI: 10.1007/s12229-013-9123-x

[12] Food and Agriculture Organization (FAO). Future fibres [Internet]. 2019. Available from: http://www.fao.org/economic/futurefibres/fibres/sisal/en/online [Accessed: 01 February 2019]

[13] Bessadok A, Langevin D, Gouanvé F, Chappey C, Roudesli S, Marais S. Study of water sorption on modified *Agave* fibres. Carbohydrate Polymers. 2009;**76**:74-85. DOI: 10.1016/j.carbpol.2008.09.033

[14] Flores-Sahagun TH, Dos Santos LP, Dos Santos J, Mazzaro I, Mikowski A. Characterization of blue *Agave* bagasse fibers of Mexico. Composites Part A: Applied Science and Manufacturing. 2013;**45**:153-161. DOI: 10.1016/j.compositesa.2012.09.001

[15] Martin AR, Martins MA, Mattoso LH, Silva OR. Caracterização química e

estrutural de fibra de sisal da variedade *Agave sisalana*. Polímeros: ciência e tecnologia. 2009;**19**(1):40-46

[16] Yang X, Cushman JC, Borland AM, Edwards EJ, Wullschleger SD, Tuskan GA, et al. A roadmap for research on crassulacean acid metabolism (CAM) to enhance sustainable food and bioenergy production in a hotter, drier world. The New Phytologist. 2015;**207**(3):491-504. DOI: 10.1111/nph.13393

[17] Goldbeck R, Ramos M, Pereira G, Maugeri-Filho F. Cellulase production from a new strain *Acremonium strictum* isolated from the Brazilian biome using different substrates. Bioresource Technology. 2013;**128**:797-803. DOI: 10.1016/j.biortech.2012.10.034

[18] Viel AM, Pereira AR, Neres WE, Dos Santos L, Oliva Neto P, Souza EB, et al. Effect of *Agave sisalana* Perrine extract on the ovarian and uterine tissues and fetal parameters: Comparative interventional study. International Multispeciality Journal of Health. 2017;**3**:129-138

[19] Rodríguez-Garay B. Somatic embryogenesis in *Agave* spp. In: Loyola-Vargas V, Ochoa-Alejo N, editors. Somatic Embryogenesis: Fundamental Aspects and Applications. Cham: Springer; 2016. DOI: 10.1007/978-3-319-33705-0_16

[20] Chen PY, Kuo YC, Chen CH, Kuo YH, Lee CK. Isolation and immunomodulatory effect of homoisoflavones and flavones from *Agave sisalana* Perrine ex Engelm. Molecules. 2009;**14**:1789-1795. DOI: 10.3390/molecules14051789

[21] Debnath M, Mukeshwar P, Sharma R, Thakur GS, Lal P. Biotechnological intervention of *Agave sisalana*: A unique fiber yielding plant with medicinal property. Journal of Medicinal Plant Research. 2010;**4**:177-187

[22] Carneiro F d S, de Oliveira Domingos Queiroz SR, Rodrigues Passos A, Neves do Nascimento M, Souza dos Santos K. Embriogênese somática em *Agave sisalana* Perrine: indução, caracterização anatômica e regeneração. Pesquisa Agropecuária Tropical. 2014;**44**:3

[23] Sidana J, Singh B, Sharma OP. Saponins of *Agave*: Chemistry and bioactivity. Phytochemistry. 2016;**130**:22-46. DOI: 10.1016/j.phytochem.2016.06.010

[24] Rajaud A, de Noblet-Ducoudré N. Tropical semi-arid regions expanding over temperate latitudes under climate change. Climatic ChangeClimatic Change. 2017;**144**(4):703-719. DOI: 10.1007/s10584-017-2052-7

[25] Sharma S, Varshney VK. Chemical analysis of *Agave sisalana* juice for its possible utilization. Acta Chimica and Pharmaceutica Indica. 2012;**2**:60-66

[26] IBGE. Instituto Brasileiro de Geografia e Estatística. Sistema IBGE de Recuperação Automática [Internet]. 2015. Available from: http://www.sidra.ibge.gov.br/ [Accessed: 27 February 2015]

[27] Instituto Brasileiro de Geografia e Estatística (IBGE). Levantamento Sistemático da Produção Agrícola [Internet]. 2011. Available from: http://www.sidra.ibge.gov.br/ [Accessed: 2011-08-01]

[28] Mattoso LHC, Ferreira FC, Curvelo AAS. In: Leão AL, Carvalho FX, Frollini E, editors. Lignocellulose-Plastic Composites. São Paulo, Brazil: USP and UNESP; 1997

[29] Silva ORR, Beltrão NEM. O agronegócio do sisal no Brasil. Campina Grande, Brasil: Embrapa-CNPA; 1999

[30] Silva ORRF, Coutinho WM, Cartaxo WV, Sofiatti V, Filho JLS, Carvalho OS,

Costa LD. Cultivo do Sisal no Nordeste Brasileiro. Ministério da Agricultura, Pecuária e Abastecimento, Circular técnica. 2008; 123

[31] Santos POD, Silva ACMD, Corrêa ÉB, Magalhães VC, Souza JTD. Additional species of *Aspergillus* causing bole rot disease in *Agave sisalana*. Tropical Plant Pathology. 2014;**39**(4):331-334. DOI: 10.1590/S1982-56762014000400008

[32] Wallace GB, Dieckmahns EC. Bole rot in sisal. East African Agricultural. 1952;**18**:24-29. DOI: 10.1080/03670074.1952.11664819

[33] Lima EF, Moreira JDAN, Batista FAS, Silva ORRF, Farias FJC, Araújo AE. Podridão vermelha do tronco do sisal (*Agave sisalana* Perrine.) causada por *Botryodiplodia theobromae* pat. Revista de Oleaginosas e Fibrosas. 1998, 1998;**2**:109-112

[34] Barbosa LO, Lima JS, Magalhães VC, Gava CAT, Soares ACF, Marbach PAS, et al. Compatibility and combination of selected bacterial antagonists in the biocontrol of sisal bole rot disease. Biological Control. 2018;**63**(4):595-605. DOI: 10.1007/s10526-018-9872-x

[35] SÁ JO. Controle biológico da podridão vermelha do sisal (*Agave sisalana* Perrine) com *Trichoderma* spp. e Actinobactérias [MSc thesis]. Cruz das Almas: Universidade Federal do Recôncavo da Bahia; 2019

[36] Abreu KCLDM. Epidemiologia da podridão Vermelha do Sisal no Estado da Bahia [MSc thesis]. Cruz das Almas: Universidade Federal do Recôncavo da Bahia; 2010

[37] Duarte EAA, Damasceno CL, Oliveira TASD, Barbosa LDO, Martins FM, Silva JRDQ, et al. Putting the mess in order: A*spergillus welwitschiae* (and not *A. niger*) is the etiological agent of sisal bole rot disease in Brazil. Frontiers in Microbiology. 2018;**9**:1-21. DOI: 10.3389/fmicb.2018.01227

[38] Machado AA. Sobre a Ocorrência de uma Nova Moléstia do Agave na Paraíba. Technical report. Relatório de uma viagem realizada no município de Campina Grande; 1951

[39] Medina JC. O sisal. Secretaria da Agricultura do Estado de São Paulo. São Paulo, Brazil; 1954

[40] Coutinho WM, Suassuna ND, Luz CM, Suinaga FA, Silva ORRF. Bole rot of sisal caused by *Aspergillus niger* in Brazil. Fitopatologia Brasileira. 2006;**31**:605-605. DOI: 10.1590 S0100-41582006000600014

[41] Varga J, Frisvad JC, Kocsubé S, Brankovics B, Tóth B, Szigeti G, et al. New and revisited species in *Aspergillus* section *Nigri*. Studies in Mycology. 2011;**69**:1-17. DOI: 10.3114/sim.2011.69.01

[42] Ismail MA. Incidence and significance of black aspergilli in agricultural commodities: A review, with a key to all species accepted to-date. European Journal of Biological Research. 2017;**7**:207-222. DOI: 10.5281/zenodo.834504

[43] Pitt JL, Hocking AD. Fungi and Food Spoilage. Cambridge: Chapman & Hall; 1997. DOI: 10.1007/978-0-387-92207-2

[44] Samson RA, Houbraken JAMP, Kuijpers AFA, Frank MJ, Frisvad JC. New ochratoxin A or sclerotium producing species in *Aspergillus* section *Nigri*. Studies in Mycology. 2004;**50**:45-61

[45] Varga J, Kocsubé S, Tóth B, Frisvad JC, Perrone G, Susca A, et al. *Aspergillus brasiliensis* sp. nov., a biseriate black *Aspergillus* species with world-wide distribuition. International Journal of Systematic and Evolutionary

Microbiology. 2007;**57**:1925-1932. DOI: 10.1099/ijs.0.65021-0

[46] Noonim P, Mahakarnchanakul W, Varga J, Frisvad JC, Samson RA. Two novel species of *Aspergillus* section *Nigri* from Thai coffee beans. International Journal of Systematic and Evolutionary Microbiology. 2008;**58**:1727-1734. DOI: 10.1099/ijs.0.65694-0

[47] Oliveri C, Torta L, Catara VA. Polyphasic approach to the identification of ochratoxin A-producing black *Aspergillus* isolates from vineyards in Sicily. International Journal of Food Microbiology. 2008;**127**:147-154. DOI: 10.1016/j.ijfoodmicro.2008.06.021

[48] Perrone G, Varga J, Susca A, Frisvad JC, Stea G, Kocsubé S, et al. *Aspergillus uvarum* sp. nov., an uniseriate black *Aspergillus* species isolated from grapes in Europe. International Journal of Systematic and Evolutionary Microbiology. 2008;**58**:1032-1039. DOI: 10.1099/ijs.0.65463-0

[49] Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CHW, et al. Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology. 2014;**78**:141-173. DOI: 10.1016/j.simyco.2014.07.004

[50] Hubka V, Kolarik M. Beta-tubulin paralogue tubC is frequently misidentified as the benA gene in *Aspergillus* section *Nigri* taxonomy: Primer specificity testing and taxonomic consequences. Persoonia. 2012;**29**:1-10. DOI: 10.3767/003158512X658123

[51] Embrapa. Cultivo do sisal [Internet]. 2010. Available from http://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Sisal/CultivodoSisal/doencas.html [Accessed: 25 May 2010]

[52] Suinaga FA, Silva ORRF, Coutinho WM. Cultivo de sisal na região Semi-árida do Nordeste Brasileiro. Campina Grande, Brazil; 2006. p. 44

[53] Magalhães VC, Barbosa LO, Andrade JP, Soares ACF, de Souza JT, Marbach PAS. *Burkholderia* isolates from a sand dune leaf litter display biocontrol activity against the bole rot disease of *Agave sisalana*. Biological Control. 2017;**112**:41-48. DOI: 10.1016/j.biocontrol.2017.06.005

[54] Baker KF. Evolving concepts of biological control of plant pathogens. Annual Review of Phytopathology. 1987;**25**:67-85

[55] Mueller UG, Sachs JL. Engineering microbiomes to improve plant and animal health. Trends in Microbiology. 2015;**23**:606-617. DOI: 10.1016/j.tim.2015.07.009

[56] do Carmo CO, Tavares PF, da Silva RM, Damasceno CL, Sá JO, Soares ACF. Fatores que afetam a sobrevivência de *Aspergillus niger* e sua relação com a podridão vermelha do caule do sisal. Magistra. 2018;**29**:144-153