Original Research Article

Diagnosis and biocontrol of *Sporisorium scitamineum* associated withwhip smut sugarcane

Abstract

Aim: Whip smut of sugarcane is the most serious and widely spread disease of sugarcaneand causes a significant reduction in cane quantity and quality.This work aimed to assess the macroscopic and microscopic characteristics and the variability between isolates of *Sporisorium scitamineum* causal agent of smut in sugarcane.

Materials and Methods: Thefungus was isolated on PDA medium. The morphological characters were described based on the growth diameter, texture, and colony colors. The microscopic description focused on the spore's size from medium and teliospores from whips. **Results:**The colony appeared with white mycelial cotton and a yeast-shaped mycelial colony. In vitro, control of *Sporisorium scitamineum* isolates with endophytic fungi of *Macrophomina phaseolina* and *Trichoderma viride*. The results showed that the two fungi could inhibit the growth of *S. scitamineum*. A pathogenicity test was carried out on sugarcane cuttings by inoculating the buds with spore suspension of *S. scitamineum*, which showed a pathogenic effect ranging from 50–100%. Also, other sugarcane cuttings were inoculated by the pathogen and *Trichoderma viride* as a biocontrol agent that inhibited the pathogen growth completely in four strains.

Conclusion: *Trichoderma viride* is the safely biocontrol agent against *S. scitamineum.*

Comment [BZN1]: Acronyms on the abstract??

Keywords: Sugarcane; *Sporisorium scitamineum*; Whip smut

1. Introduction

The greatest commercial crop in the world is sugarcane, *Saccharum officinarum* L., which is grown on approximately 26.27 million hectares in more than 120 countries and yields 1.90 billion tonnes annually. According to the FAO[1], sugarcane is the source of almost 80% of the sugar consumed worldwide. According to Almazan et al. [2], the annual harvest of sugarcane produces 75,000 million calories and hundreds of tons of green matter per hectare. The economy of Upper Egypt is mostly reliant on sugarcane cultivation. Many people depend on the income from sugarcane growing, and any disruptions to that region would have a ripple effect through the economy. The total amount of sugarcane harvested between 1994 and 2016 was 15532.49 metric tons. Upper Egypt is one of many tropical and temperate locations that cultivate sugarcane. Spring and autumn are the best times to plant it. The months of February and March are used for spring planting, whereas the months of September and October are used for fall planting [3].

The sugarcane crop needs 12 to 14 months to reach maturity and be harvested, and during this time, it is subject to a variety of biotic and abiotic stresses. Pathogens and insect pests have the ability to reduce productivity by up to 20% on an individual basis [4, 5]. Fungal pathogens are the most problematic biotic agents. According to Subhani et al. [6], more than 100 fungus have been identified as sugarcane disease-causing agents. Whip smut, red rot, leaf blast, sugarcane mosaic virus, pineapple disease, ratoon stunting disease, leaf scald, mottled stripe, pokkahboeng, and wilt are the most serious sugarcane diseases [7].

The whip smut caused by *Sporisorium scitamineum* (Phylum: Basidiomycota, Order: Ustilaginales) is considered the most serious and widely spread disease of sugarcane, known to affect both qualitative and quantitative components causing substantial economic losses[8, 9].

Comment [BZN2]: Consistency in writing "tonnes" and "tons"

Smut is mostly dispersed by wind, although it can also spread through infected agricultural equipment and propagation materials. According to Comstock [10], infection with soil teliospores can also result in the disease. Smut sori are formed in response to infection in a variety of plant species. According to Ferreira and Comstock [4], in sugarcane, the pathogen changes the development of the plant shoot to generate a sorus or "whip." The sorus is now considered to be an elongated internode rather than an inflorescence as Glassop et al. [11] showed that none of the blooming genes are expressed during sorus production [12]. The sorus can be anything between a few millimeters and 1.5 meters long [10]. It consists of a narrow cylinder of teliospores encircling a central core of parenchymatous/fibrovascular elements [4]. The sorus grows from the tip of the stalks, is unbranched, and can be pencil-thin (but it can also be thicker occasionally) [13]. Infested areas suffer extremely high air spore densities as a result of teliospores released by sick sensitive crops. According to estimates made by Lee-Lovick in 1978 [14], smut sori produce 108 to 109 teliospores daily. Teliospores can survive in dry soil for more than six months before becoming inactive, but soil moisture prevents soil survival and reduces viability to two to three months [15].

Teliospores created in the smut whip are used to spread sugarcane smut. Water is necessary for the teliospores to germinate, whether they are on the plant or in the soil [16]. They germinate, make promycelium, and then go through meiosis to produce four haploid sporidia. Due of the bipolar nature of sugarcane smut, two distinct sporida mating types are produced. Two sporida from various mating types must unite to create a dikaryon in order for infection to take place. Then, this dikaryon creates hyphae that penetrate the sugarcane plant's bud scales and infect the meristematic tissue. In order to create its teliospores, the fungus colonizes newly formed floral structures as it develops within the meristematic tissue [17]. A whip-like structure

called a sorus develops between the leaf sheaths from the flowering structures, which are typically regular grass arrows. When dehydrated, the thin silvery peridium (the host tissue) that first covers it easily peels aside to reveal the sooty black-brown teliospores. The cycle then continues as these teliospores are distributed by the wind. The spores are spherical, sub-ovoid, and reddish brown in color. They can be smooth or somewhat echinulate and have tiny spines or prickles. The range of sizes is 6.5 to 8 um.

2. Materials and methods

2.1 Collection of smut samples

We collected samples (13 samples) fromthe whip smut of sugar cane of the commercial variety grown in Upper Egypt in(Qena Governorate, Deshina.Center, Al-Azazia region) in the month of May 2023.

2.2 Isolation of the causal agent of smut from sugarcane

Two methods were done for isolation of *Sporisorium scitamineum*, dilution-plateand direct-plate methods on PDA medium at 28 °C for 7 days, Abdel-Fatth et al. [18].

Comment [BZN3]: Write in full on the first mention

2.3 Microscopic Observations

For microscopic observation, samples of floral structure with smut were detected and imaged by light microscope. Teliospores were examined directly from the samples to glass slides. The isolated strains were cultured on PDA medium for 7 days at 28°C for observing sporidia. Teliospores and sporidia shape, color, and sizes for each isolated strain were analyzed.

Comment [BZN4]: The methodology has no citations

Comment [BZN5]: What was used to measure the teliospore sizes?

2.4 Antagonistic test of *Macrophomina phaseolina*and *Trichoderma viride* against smut fungus with direct opposition method

The isolates of *Sporisorium scitamineum* and endophytic fungiwere put together on a petri dish containing PDAmedium within 3 centimeters lengthand incubated in the room temperature of 28-30°C duringa week. For a treatment of control, pieces of isolate were put on the petri dish without endophytic fungus *Macrophomina phaseolina* or *Trichoderma viride*. The treatment was repeated for 3times. The observed variable was colony radiusthat grows to the direction of endophytic fungi.Formulation of growth inhibition (I) of pathogenic colony of endophytic fungi by Sharfuddin andMohanka[19]:I = [(r1-r2)/r1] x 100%, I= growth inhibition of *S. rolfsii* colony (%); r1= colony radius of *Sporisorium scitamineum* grows in the control (cm), r2= colony radius of *Sporisorium scitamineum* grows to the direction of*Macrophomina phaseolina* and *Trichoderma viride*(cm).

Comment [BZN6]: This could be rephrased to "3cm apart", which makes more sense than "3cm in length"

2.5 Pathogenicity Test

Thirteenfree-disease-sugarcane cuttings were used to test the virulence of the smut pathogen isolates. Cuttings with the height of 15 cm were used for the inoculation of the smut pathogen and others for the pathogen and *Trichoderma viride*, according to Yan et al. [20] with minor modifications. The isolated strains werecultured on PDA medium at 28 °Cfor 7 days, then re-suspending in 20 mL of distilled water. Half mL of a suspension strains was inoculated into the buds of cuttings of sugarcane.Other thirteen cuttings were inoculated with the isolated strains and *Trichoderma viride* as a biocontrol agent.Free inoculation cutting was used as the negative control. Re-isolation from infected tissue of cuttings were carried out according to Koch's postulates.

Comment [BZN7]: Name the variety

Comment [BZN8]: The inoculation by the *S. scitamineum* and the other prospectively antagonistic fungi should be explained. ie was it done at the same time? How was it done?

The way the inoculation was done should explain if the treatments inhibited the initial growth of the pathogen or it treated the infected sugarcane cuttings.

How did you test if the inoculation of the cuttings with the pathogen was successful? How do you know that the treated sugarcane was actually infected in the first place?

Comment [BZN9]: Which inoculation method did you use? And cite its reference

3. Results

3.1 Mycobiota isolated from infected sugarcane

Thirteen sugarcane samples were collected from farms of Qena, Egypt. Nine species of fungi were isolated from the thirteen samples using dilution- and direct plate methods on PDA medium at 28 °C for seven days. *Sporisoriumscitamineum* was the most common species isolated from the two isolation methods, with 1.56cfu/ml and total count 31/52 segments of sugarcane. *Alternaria alternata* was the second isolated species in the direct-plate method, with a total count 6/52 segments of sugarcane. (Figure 1 a and b).

Comment [BZN10]: The 13 samples: were they of a similar variety or not? What are the vulnerability indexes of the varieties? What was the purpose of collecting 13 sugarcane samples?

Comment [BZN11]: This is methodology

3.2 Morphology of *Sporisorium scitamineum*

Teliospores of *S. scitamineum* were characterized from the whips smut. They were globose, with thicked walled and brown in the colour. They ranged in the diameter from 5.1 – 13.1µm. *S. scitamineum*sporodia were described after inoculation on PDA medium for seven days at 28 °C. They showed ovate to cylindrical structure with micro- and macro-spores, measured in the range of (width, length)1.4–36.5µm. (Figure 2 and 3).

Comment [BZN12]: What did you use to measure the spore diameters?

Comment [BZN13]: This part is falls under methodology

Comment [BZN14]: If it is width and length then it cannot be a range

Comment [BZN15]: 1.4µm is too small for a mycelial length, especially after incubating for 7 days, also, especially since the spore size ranges from 5.1 – 13.1µm. Revise the typo

3.3 Antagonistic potential of *Trichoderma viride* and *Macrophomina phaseolina* against *S. scitamineum*

The potential of two fungal species *Trichoderma viride* and *Macrophomina phaseolina* to inhibit the growth of thirteen isolates of *S.scitamineum* was studied. The highest inhibition percentage was observed by *Trichoderma viride*agaist isolate no. 9 with percentage 66.6%. (Table 1 and Figure4). *Macrophomina phaseolina* exhibited inhibition in the growth of all tested isolates with different percentage 8.3–50%.

Comment [BZN16]: Is it 13 isolates or 13 samples? Are these isolates of the same strain or of different strains?

3.4 Pathogenic effect of *Sporisoriumscitamineum*

Eight isolates of *S. scitaminum* inoculated the buds of sugarcane cuttings. The pathogenic effect of tested isolates was evaluated after seven days of incubation at 28 °C in the dark. Another experiment was carried out under the same conditions by inoculating the buds of sugarcane cuttings with the *S. scitaminum*isolate and *Trichoderma viride*. Five isolates from *S. scitaminum* showed highly virulent smut, ranged 90 – 100%, on sugarcane cuttings, and three isolates exhibited moderate virulent smut at 50%. Pathogenicity activity of *S. scitaminum*isolates on sugarcane cuttings inoculated by *Trichoderma viride* was tested. It observed that four isolates were completely inhibited and not exhibited smut on cuttings at 0%, and the other four tested isolates showed smut on inoculated cuttings with 30% (low virulent). The pathogenic effect of the eight *S. scitaminum*isolates was observed on the sugarcane leaves. Two isolates were highly virulent against leaves (90%), four isolates were low virulent (20-30%), and two isolates were not pathogenic to the tested leaves (0%). (Figure 5, 6 and Table 2).

Comment [BZN17]: Were the cuttings from the same variety? If it was different varieties, did they have the same susceptibility index?

Comment [BZN18]: Does this mean the isolates were highly virulent or the sugarcane was very susceptible or the pathogen found favourableconditions.
How did you measure the 90 – 100%

Comment [BZN19]: Smut is a systemic pathogen and the delayed exhibition of symptoms does not mean 0 infection. Methods of testing systemic infection include using PCR. For this study, which method did you use to detect the pathogen in the inoculated plants

4. Discussion

In a comparison of mycobiota isolated from infected sugarcane by using dilution- and direct-plate methods on PDA medium at 28 ±2°C, it was found that the total fungal populations in case of dilution–plate method were so low than those of direct–plate method. *Sporisoruim scitamineum* also appeared in a high population and was parallel to total fungal counts (1.56 CFU/ml in dilution method and 31 colonies/ 52 segments. *Alternaria alternata* was isolated with high total counts in the direct-plate method (7 colonies/ 52 segments) anddid not appear in the

dilution method. Some fungal species were not completely appeared on the dilution-plate method and appeared on the direct-plate method. These were *A. flavus*, *A. terreus*, *Alternaria alternata*, *Phoma* sp., and *Penicillium duclauxii*. These species appeared when direct–plate method was used.The isolates of *Sporisorium scitamineum*, an agent of smut, wereisolated and studied by Jacques-Edouard et al. [21]. The localities of Zuenoula and Ferke 2 showed the highest rates of isolation of fungal strains. The morphological and molecular variability of *Sporisorium scitamineum* isolates associated with sugarcane from Eswatini were also studied by Nkhabindze et al. [22].

Spores (teliospores) of sugarcane smut, *Sporisorium scitamineum*; they are black when together on the whip, but under the microscope, and circular in shape with a spiny membrane and were brown in colour for all the isolates. They are roughly spherical and 5.5-7.5 µm in diameter Nkhabindze et al [22]. Microscopic description of the isolates revealed that spore teliospore diameter vary between isolates, teliospore diameter rangedfrom 7.33 µm to 10.25 µm. Teliospores are circular in shape with a thick wall,thediameters of which varied according to the isolates collected Jacques-Edouard et al. [21]. Germination gives rise to a four-celled promycelium that in turn produces either hyphae or sporidia from each of the cells. Sporidia are ovoid, 6 x 2 µm and hyaline. Sporidia continue to multiply asexually by budding in a yeast-like manner. Single-cell colonies continue to grow in a yeast-like manner and may be maintained indefinitely in vitro[23].

The study's results showed that *Trichoderma viride* suppressed the growth of *S. scitamineum* to varied degrees. In the dual culture, the *Trichoderma viride* outgrew the pathogen more quickly, severely impeding its radial growth by overgrowth and creating inhibition zones. This might attest to the fact that the native Trichoderma isolates had an exceptional antagonistic effect on the

pathogen. In order to combat the sugarcane smut disease, they were discovered to be promising biocontrol agents. Numerous researchers [24, 25] have already thoroughly documented the antagonistic action of the *Trichoderma* isolate against *U. scitaminea* and other sugarcane diseases.

U. scitaminea growth was discovered to be inhibited by the *Trichoderma* isolates by three separate mechanisms: overgrowth, chemical secretion that limits pathogen growth as seen by the creation of an inhibition zone, and combining the two modes simultaneously. Accordingly, Juma et al. [24] and Suresh and Nelson [26] both underlined the highly diverse processes that allowed *Trichoderma* isolates to colonize numerous pathogens and their environments. As a result, the researchers confirmed that the smut and other pathogens can be suppressed by hyperparasitism, antibiosis, metabolite synthesis, competition for available nutrients, and combinations of two or more of the stated processes [27].

The isolate was individually injected onto mature stalks of commercial sugarcane for the pathogenicity test. Sugarcane stem cuttings that had been injected with the isolate developed tissue rot and discolouration. The degree of the fungus isolate's influence on the infected sugarcane nodes. Additionally, the results demonstrated that, when compared to the pathogen with *Trichoderma viride* stalk biocontrol, inoculating the isolate into sugarcane nodes under laboratory settings could result in a significant degree of rot and discolouration after 3 days. The results of the pathogenicity testing trial supported Koch's hypotheses and shown that the isolate of the fungus was destructive to sugarcane cuttings, whereas *Trichoderma viride* inhibited the growth of *Sporisorium scitamineum* in sugarcane stalk cuttings [28].

5. Conclusion

Trichoderma viride and *Macrophomina phaseolina* were the effective biocontrol agents that can be used to overcome the whip smut of sugarcane caused by *S. scitamineum*.

References

1. FAO. FAOSTAT, Crops: Sugarcane. 2021. Available online: http://www.fao.org/faostat/en/#data/QC (accessed on 28 July 2021).

10. Comstock JC. Seed cane transmissible diseases in sugarcane and their management. Souvenir de Presentation. InProceedings of the International Conference IS-2014, Green Technologies for Sustainable Growth of Sugar & Integrated Industries in Developing Countries, Nanning, China, 25–28 ,2014 Nov 25 (pp. 24-27)

10. Comstock JC. Smut. A Guide to Sugarcane Diseases'. (Eds P Rott, RA Bailey, JC Comstock, BJ Croft, AS Saumtally) CIRAD and ISSCT, Montpellier, France .pp. 2000:181-185.

11. Glassop D, Bonnett G, Croft B, Bhuiyan S, Aitken K, Rae A, BRUCE L. Flowering-related genes are not involved in the development of smut whip. InProc. Aust. Soc. Sugar Cane Technol 2014 Apr (Vol. 36, pp. 244-253).

12. Marques JP, Appezzato-da-Glória B, Piepenbring M, Massola Jr NS, Monteiro-Vitorello CB, Vieira ML. Sugarcane smut: shedding light on the development of the whip-shaped sorus. Annals of Botany. 2017 Mar 1;119(5):815-27.

13. Bhuiyan, S. A., Magarey, R. C., McNeil, M. D., & Aitken, K. S. Sugarcane smut, caused by *Sporisorium scitamineum*, a major disease of sugarcane: A contemporary review. Phytopathology®, 2021;111(11), 1905-1917.

14. Lee-Lovick G. Smut of sugarcane-*Ustilago scitaminea*. Review of plant pathology. 1978;57:181-188.

15. Bhuiyan S, Croft BJ, Cox MC. Survival of sugarcane smut teliospores under south east Queensland conditions. InProc Aust Sugar Cane Technol 2009 (Vol. 31, pp. 135-144).

16. Sundar AR, Barnabas EL, Malathi P, Viswanathan R, Sundar AR, Barnabas EL. A mini-review on smut disease of sugarcane caused by *Sporisorium scitamineum*. Botany InTech,. 2012 May;2014:226. pp. 107– 128.

17. Carvalho G, Quecine MC, Longatto DP, Peters LP, Almeida JR, Shyton TG, Silva MM, Crestana GS, Creste S, Monteiro-Vitorello CB. *Sporisorium scitamineum*colonisation of sugarcane genotypes susceptible and resistant to smut revealed by GFP-tagged strains. Annals of Applied Biology 169. 2016;169(3):329-341.

18. Abdel-Fattah HM, Moubasher AH, Abdel-Hafez SI. Fungus flora of root and leaf surface of broadbean cultivated in oases, Egypt. *Naturaliamonspeliensia*. Serie botanique. 1979. 27:167–177.

19. Sharfuddin C, Mohanka RE. In vitro antagonism of indigenous *Trichoderma* isolates against phytopathogen causing wilt of lentil. International journal of life science & Pharma Research. 2012;2:195-202.

2. Almazan, O.; Gonzalez, L.; Galvez, L. The sugar cane, its by-products and co-products. In Proceedings of the Third Annual Meeting of Agricultural Scientists, Réduit, Mauritius. Food and Agricultural Research Council: Réduit, Mauritius, 1999.

20. Yan M, Zhu G, Lin S, Xian X, Chang C, Xi P, Shen W, Huang W, Cai E, Jiang Z, Deng YZ. The mating-type locus b of the sugarcane smut *Sporisorium scitamineum i*s essential for mating, filamentous growth and pathogenicity. Fungal Genetics and Biology. 2016;86:1-8.

21. Jacques-Edouard YK, Didier KK, Gaston KK, Jean-Martial KKF, et al. Structure of *Sporisorium Scitamineum* Isolates, the Causative Agent of Sugarcane Smut in Côte D'ivoire. International Journal of Science and Research. 2019; 7.583 .

22. Nkhabindze B, Earnshaw DM, Ateka EM, Wanyika HN. The morphological and molecular variability of *Sporisorium scitamineum* isolates from Eswatini.2022;16(11):1253-1260.

23. CABI (2022) '*Sporisorium scitamineum* (sugarcane smut)', CABI Compendium. CABI International. doi: 10.1079/cabicompendium.55949.

24. Juma, E., Musyimi, O. A., & George, D. M. Antagonistic potential of selected fungal and bacterial isolates from rhizosphere of sugarcane variety Co421 against *sporisorium scitamineum* in Kibos, Kenya. Applied Science Report. 2018; 23(1), 7–15. DOI:10.15192/PSCP.ASR.2018.23.1.715.

25. Shailbala, V., Kumar, A., & Sharma, S. K. Management of red rot of sugarcane caused by *colletotrichumfalcatum* went. International Journal of Chemical Studies. 2019; 7(3), 5029–5039. https://www. researchgate.net/publication/294697274.

26. Suresh, N., & Nelson, R. Isolation of antagonistic fungi and evaluation of anti-fungal activity of the separated metabolite against the red rot of sugarcane pathogen. European Journal of Experimental Biology. 2016; 6 (1), 15–21. www.pelagiaresearchlibrary.com.

27. Tegene, S., Dejene, M., Terefe, H., Tegegn, G., Tena, E., & Ayalew, A. Evaluation of native *Trichoderma* isolates for the management of sugarcane smut *(Ustilago scitaminea*) in sugar plantations of Ethiopia. Cogent Food & Agriculture. 2021; 7(1), 1872853.

28. Osman, M. A. M. Isolation and identification of the causal pathogen associated with Pokkah Boeng disease on sugarcane in Upper Egypt. SVU-International Journal of Agricultural Sciences. 2021; 3(3), 30-39.

3. Elasraag YH. Economic analysis of sugarcane and sugar beet in Egypt. Zagazig Journal of Agricultural Research. 2019 Feb 22;46(1):209-15.

4. Ferreira, S.A.; Comstock, J.C. Common Names of Plant Diseases—Sugarcane; American Phytopathological Society: St. Paul, MN,USA, 1993.

5. Singh, R.S. Plant Diseases,.;Medtech: Chennai, India.10th ed, 2017; 782p.

6. Subhani MN, Chaudhry MA, Khaliq A, Muhammad F. Efficacy of various fungicides against sugarcane red rot (*Col- letotrichumfalcatum*). Int. J. Agric. Biol. 2008;10:725-7.

7. Sivanesan,A.;Waller,J.M.SugarcaneDiseases;CMIPhytopathologyPaperNo.29;CABInt. :Surrey,UK,1986;88p

8. Indi DV, Nalawade SV, Pawar N. Stable sources of whip—Smut resistance in sugarcane. In proceedings of the international symposium on new Paradigms in sugar cane. Research, Sugar cane Breeding Institute, Coimbatore, India 2012 Oct (pp. 15-18).

9. Wada AC, Anaso AB. Yield components and cane yield losses of two sugarcane varieties affected by whip smut (*Sporisorium scitaminem Sydow*) in Nigeria. J. Crop Sci. Res. 2016;1:1-5.

Figure (1):

(A): CFU/ml of species isolated from infected sugarcane with dilution-plate method on PDA medium at 28 °C for 7 days.

Fungal species	Sample number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Count
Aspergillus niger	0.03	0	0.06	0.05	0	0	0.1	0.03	0.03	0	0	0.2	0.04	0.54
Penicillium chrysogenum	0	0.01	0	0	0	0	0	0.05	0	0	0	0	0	0.06
Sporisorium scitamineum	0.15	0.2	0.01	0.2	0.3	0	0.15	0	0.05	0.1	0.1	0	0.3	1.56
Trichoderma viride	0	0	0	0	0	0.2	0	0.2	0	0	0	0	0	0.4

(B): Fungal counts of species isolated from infected sugarcane with direct-plate method on PDA medium at 28 °C for 7 days.

Fungal species	Sample number													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Total Count
Alternaria alternata	0	0	0	0	0	0	0	4	0	1	0	1	0	6
Aspergillus flavus	2	0	1	0	0	0	0	0	0	1	0	0	1	5
Aspergillus niger	1	0	0	0	0	0	2	1	0	0	0	0	1	5
Aspergillus terreus	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Penicillium duclauxii	0	0	0	4	0	0	0	0	0	0	0	0	0	4
Penicillium chrysogenum	0	0	0	0	0	0	0	0	0	1	1	0	0	2
***Phoma* sp.**	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Sporisoruim scitamineum	3	0	4	1	0	0	3	1	4	4	4	4	3	31
Trichoderma viride	0	0	0	1	0	0	0	0	0	0	0	0	0	1

Figure (2): Morphology of *Sporisorium scitamineum*teliospores from several collected isolates using light microscope 40X.

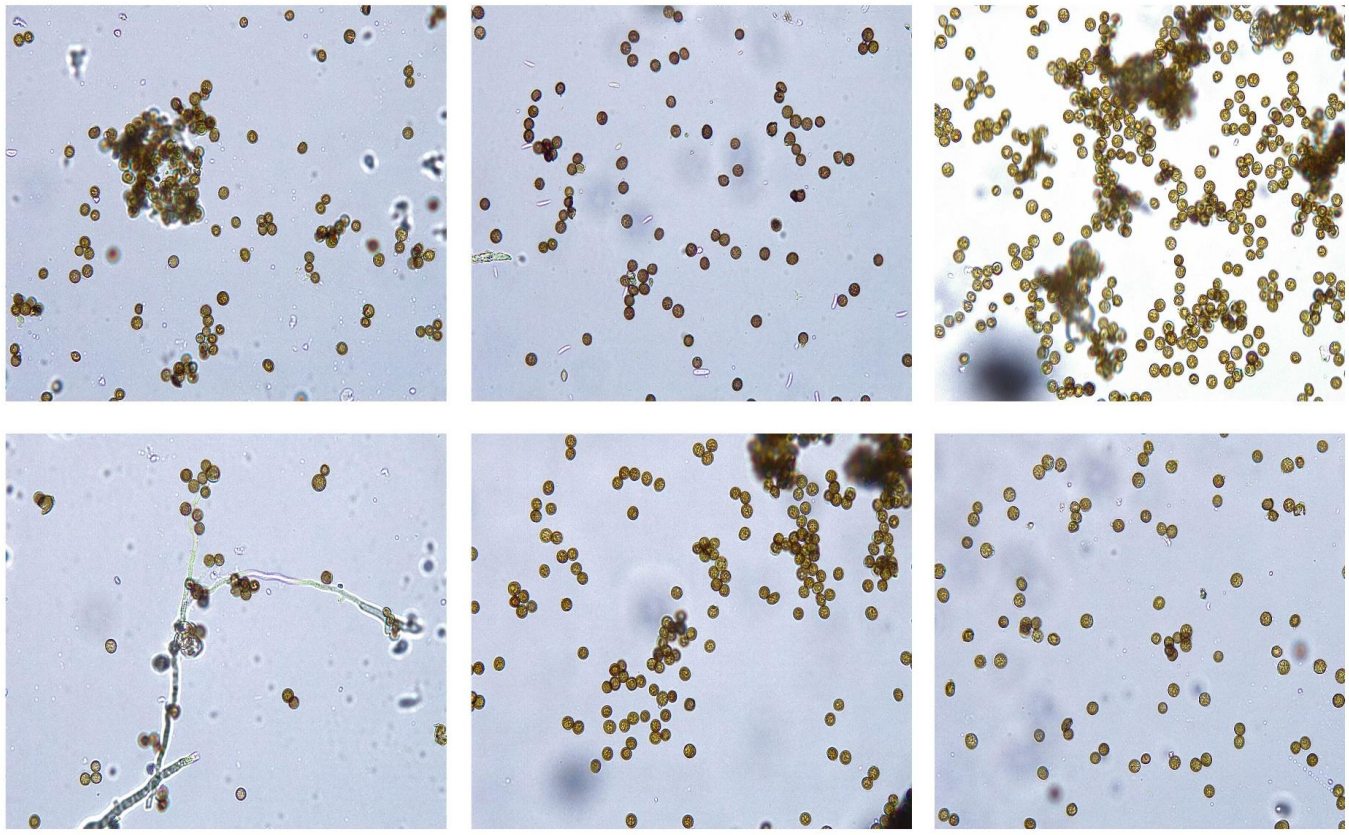

Figure (3): Microscopic characterization of *Sporisorium scitamineum*sporodia after 7 days on PDA medium at 28 °C

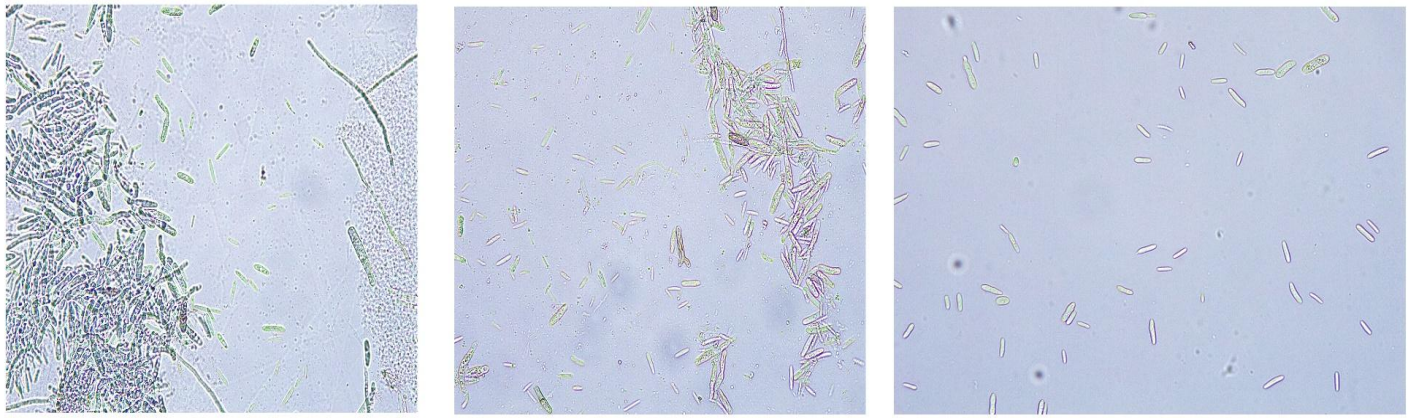

Figure (4): Antagonism between *S.scitamineum* isolates and biocontrol fungi (*Trichoderma viride* and *Macrophomina phaseolina*); the first column reprsented control of *S.scitamineum* isolates, the second column biocontrol of *S.scitamineum* by *Macrophomina phaseolina*, and the third colum showed biocontrol of *S.scitamineum* by *Trichodermaviride.*

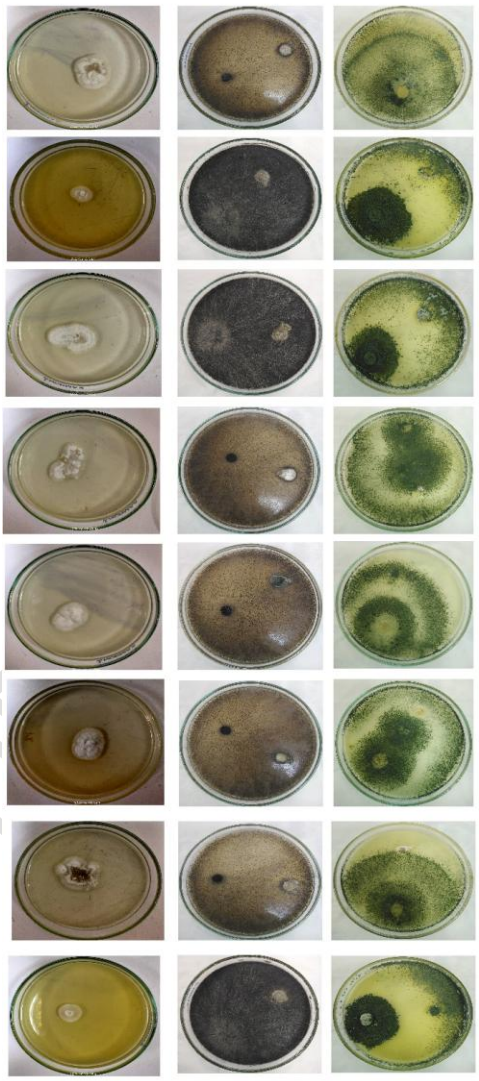

Figure (5): Pathogenicity test on sugarcane inoculated by the pathogen (the left in each image), with the pathogen and biocontrol of *Trichoderma viride* (the right one), and control was the latest image.

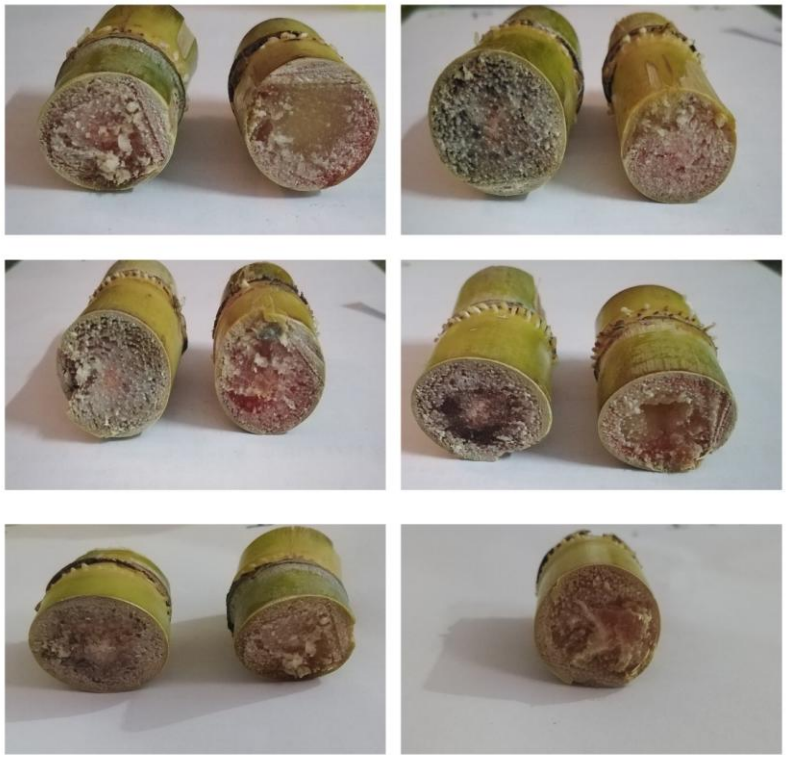

Figure (6):Pathogenicity activity of *S.scitamineum* on sugarcane leaves, from left to right (non-pathogenic, low virulent, highly virulent).

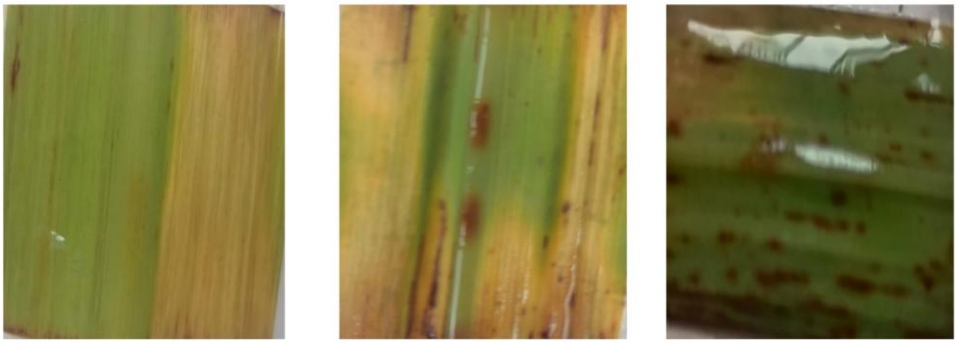

Table (1): Percentage of the growthinhibition % of tested *S. scitamineum*by biocontrol fungi *Trichoderma viride* and *Macrophomina phaseolina*

Isolate number	control	*Macrophomina phaseolina* (%)	*Trichoderma viride* (%)
1	2.1	33.3	54.5
3	1.15	8.3	33.3
8	2.4	50	18.8
9	1.85	21.1	66.6
10	1.65	29.4	46.7
11	1.9	31.6	54.5
12	2.3	43.5	40
13	1.35	21.4	33

Table (2): Pathogenic activity of *S. scitamineum*on sugarcane cuttings and leaves.

Isolate number	Pathogenicity level of *S. scitamineum*on sugarcane cuttings %	Pathogenicity level of *S. scitamineum*on sugarcane cuttings inoculated by *Trichoderma viride*%	Pathogenicity level of *S. scitamineum* on sugarcane leaves	Diameter of *S. scitamineum*teliospores	*S. scitamineum* Sporodia (W – L)
1	50	30	0	6.4 – 8.9	1.4 – 13.4
3	100	0	20	5.1 – 8.5	2.6 – 15.7
8	100	0	90	8.1 – 8.9	1.8 – 13.9
9	95	0	90	7.6 – 8.9	1.8 – 36.5
10	50	0	30	6.3 – 7.7	2.8 – 8.1
11	50	30	20	7.4 – 13.1	2.9 – 17.3
12	95	30	30	8 – 11.4	4 – 10.5
13	90	30	0	6.3 – 10.2	5.7 – 21.9

H: High= infected more than 75% of the cutting or leaf; M: Moderate= 50–75%; L: Low= less than 50%.