

## **Diagnosis and biocontrol of *Sporisorium scitamineum* associated with whip smut sugarcane**

### **Abstract**

**Aim:** Whip smut of sugarcane is the most serious and widely spread disease of sugarcane and 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:** The fungus was isolated on **Potato Dextrose Agar (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 *S. 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*.

**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 tons 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 *Sporisoriumscitamineum* (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].

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 10<sup>8</sup> to 10<sup>9</sup> 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 to the bipolar nature of sugarcane smut, two distinct sporidia mating types are produced. Two sporidia 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 awns. 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  $\mu\text{m}$ .

## **2. Materials and methods**

### **2.1 Collection of smut samples**

We collected samples (13 samples) from the whip smut of sugar cane of the commercial variety grown in Upper Egypt in (Qena Governorate, Doshina 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 *S.scitamineum*, dilution-plate and direct-plate methods on **Potato Dextrose Agar (PDA)** medium at 28 °C for 7 days, Abdel-Fatth et al. [18].

### **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 measured by **Leica DM1000 microscope**[19].

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

The isolates of *S.scitamineum* and endophytic fungi were put together on a petri dish containing PDA medium within 3 cm apart and incubated in the room temperature of 28-30°C for a week. For a control, disc of isolate was put on the petri dish without endophytic fungus *Macrophominaphaseolina* or *Trichodermaviride*. The treatment was repeated three times. The observed variable was colony radius that grows to the direction of endophytic fungi. Formulation of growth inhibition (I) of pathogenic colony of endophytic fungi by Sharfuddin and Mohanka [20]:  $I = [(r_1 - r_2) / r_1] \times 100\%$ , I = growth inhibition of *S. rolfsii* colony (%); r1 = colony radius of *S.scitamineum* grows in the control (cm), r2 = colony radius of *S.scitamineum* grows to the direction of *Macrophominaphaseolina* and *Trichodermaviride* (cm).

## 2.5 Pathogenicity Test

Eight free-disease-sugarcane cuttings of *Saccharum officinarum* L. 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 *Trichodermaviride*, according to Yan et al. [21] with minor modification, the two strains of *S.scitamineum* and *T. viride* were inoculated in the bud at the same time. Cuttings of sugarcane were surface disinfected using 5% sodium hypochlorite for 5 min then washed by sterilized distilled water and allowed to dry then inoculated by paste spore suspension [22]. The isolated strains of *S. scitamineum* were recultured on PDA medium at 28 °C for 7 days, then one disc of spores (10 mm) re-suspending in 20 mL of distilled water. Half mL of a suspension strains was inoculated into the buds of cuttings of sugarcane by panting then air dried under ambient temperature. Other eight cuttings were inoculated with the isolated strains and *Trichodermaviride* 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.

### 3. Results

#### 3.1 Mycobiota isolated from infected sugarcane

Thirteen sugarcane samples of *Saccharum officinarum* L. were collected randomly from farms of Qena, Egypt, the variety of sugarcane samples was identified by Ahmed A.A. Mahmoud in Plant Pathology Research Institute. Nine species of fungi were isolated from the thirteen samples using dilution- and direct plate methods. *S.scitamineum* was the most common species isolated from the two isolation methods, with 1.56cfu/ml and total count 31/52 segments of sugarcane. *Alternariaalternata* was the second isolated species in the direct-plate method, with a total count 6/52 segments of sugarcane. (Figure 1 a and b).

#### 3.2 Morphology of *S.scitamineum*

Teliospores of *S. scitamineum* were characterized from the whips smut. They were globose, with thickened walls and brown in colour. They ranged in diameter from 5.1 – 13.1µm. The sporidia showed ovate to cylindrical structure with micro- and macro-haploid cells, measured in the range of (width, length) 1.4–36.5µm. (Figure 2 and 3). All measurements were carried out by using Leica DM1000 microscope calibrated by a standard graduated slide [with graduation 1 mm].

#### 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 eight isolates of *S.scitamineum* that isolated from thirteen samples of sugarcane was studied. The highest inhibition percentage was observed by *Trichoderma*

*viride* against isolate no. 9 with 66.6% (Table 1 and Figure4). *Macrophomina phaseolina* exhibited inhibition in the growth of all tested isolates with different ranges 8.3–50%.

### 3.4 Pathogenic effect of *S.scitamineum*

Eight isolates of *S. scitamineum* inoculated the buds of sugarcane cuttings of the same variety *S. officinarum*. 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. scitamineum* isolate and *Trichodermaviride*. Five isolates from *S. scitamineum* showed highly virulent smut, ranged 90 – 100%, on sugarcane cuttings (the percentage of smut appeared on the tested cutting), and three isolates exhibited moderate virulent smut at 50%. Pathogenicity activity of *S. scitamineum* isolates on sugarcane cuttings inoculated by *Trichodermaviride* was tested and the observation was recorded after one week of incubation. 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. scitamineum* 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).

## 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. *S.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. *Alternariaalternata* was isolated with high total counts in the direct-plate method (7 colonies/ 52 segments) and did 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*, *Alternariaalternata*, *Phoma* sp., and *Penicilliumduclauxii*. These species appeared when direct-plate method was used. The isolates of *S.scitamineum*, an agent of smut, were isolated and studied by Jacques-Edouard et al. [23]. The localities of Zuenoula and Ferke 2 showed the highest rates of isolation of fungal strains. The morphological and molecular variability of *S. scitamineum* isolates associated with sugarcane from Eswatini were also studied by Nkhabindze et al. [24].

Spores (teliospores) of sugarcane smut, *S.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  $\mu\text{m}$  in diameter Nkhabindze et al [24]. Microscopic description of the isolates revealed that spore teliospore diameter vary between isolates, teliospore diameter ranged from 7.33  $\mu\text{m}$  to 10.25  $\mu\text{m}$ . Teliospores are circular in shape with a thick wall, the diameters of which varied according to the isolates collected Jacques-Edouard et al. [23]. 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  $\mu\text{m}$  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 [25].

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 [26, 27] 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. [26] and Suresh and Nelson [28] 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 [29].

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 *Trichodermaviride* 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 *Trichodermaviride* inhibited the growth of *S.scitamineum* in sugarcane stalk cuttings [30].

## 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*.

## Competing Interests

Authors have declared that no competing interests exist.

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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													Total Count
	1	2	3	4	5	6	7	8	9	10	11	12	13	
<i>Aspergillusniger</i>	0.03	0	0.06	0.05	0	0	0.1	0.03	0.03	0	0	0.2	0.04	0.54
<i>Penicilliumchrysogenum</i>	0	0.01	0	0	0	0	0	0.05	0	0	0	0	0	0.06
<i>Sporisoriumscitamineum</i>	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
<i>Trichodermaviride</i>	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													Total Count
	1	2	3	4	5	6	7	8	9	10	11	12	13	
<i>Alternariaalternata</i>	0	0	0	0	0	0	0	4	0	1	0	1	0	6
<i>Aspergillus flavus</i>	2	0	1	0	0	0	0	0	0	1	0	0	1	5
<i>Aspergillusniger</i>	1	0	0	0	0	0	2	1	0	0	0	0	1	5
<i>Aspergillusterreus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1
<i>Penicilliumduclauxii</i>	0	0	0	4	0	0	0	0	0	0	0	0	0	4
<i>Penicilliumchrysogenum</i>	0	0	0	0	0	0	0	0	0	1	1	0	0	2
<i>Phoma sp.</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	1
<i>Sporisoriumscitamineum</i>	3	0	4	1	0	0	3	1	4	4	4	4	3	31
<i>Trichodermaviride</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	1

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

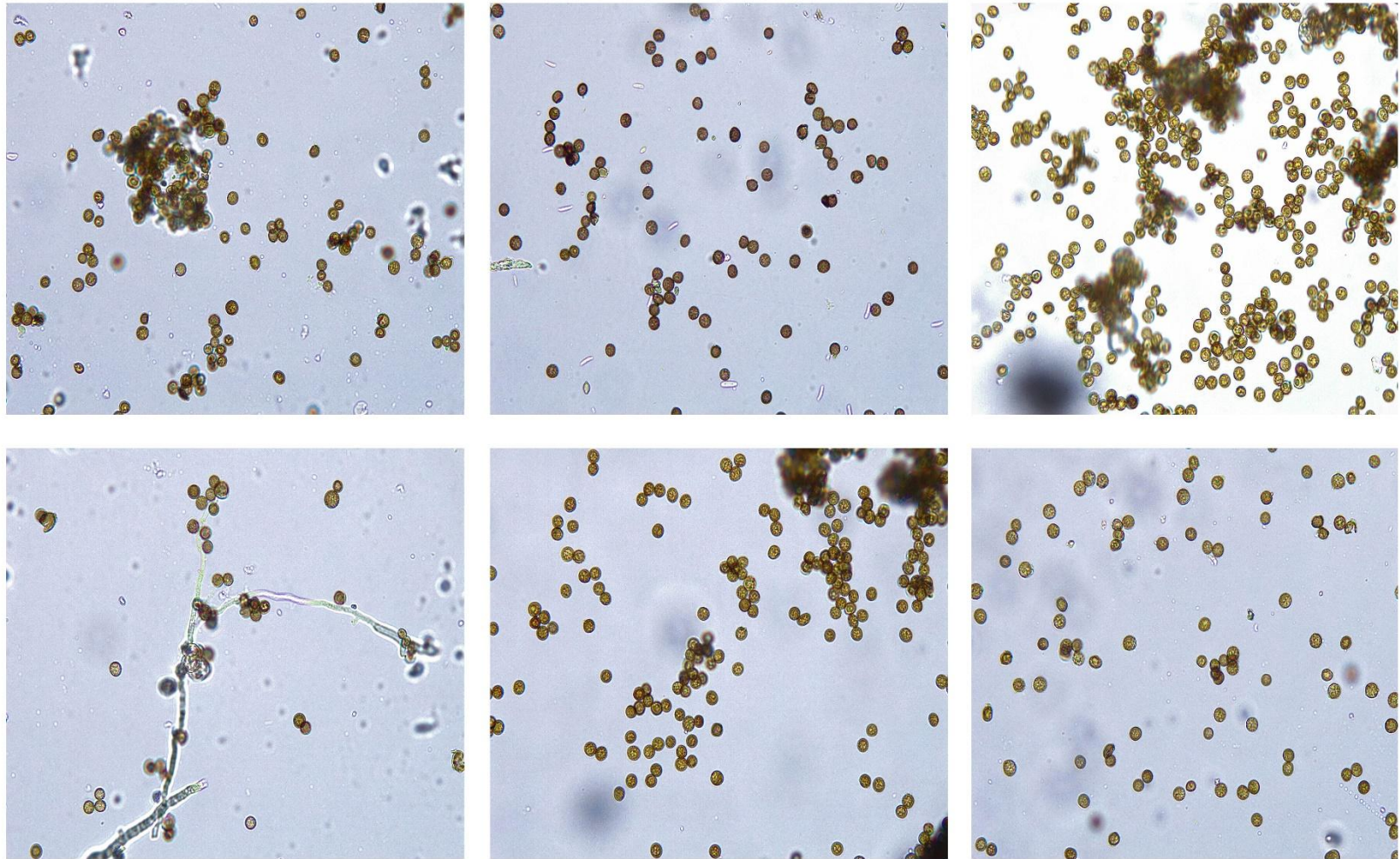


Figure (3): Microscopic characterization of *Sporisoriumscitamineum* sporodia after 7 days on PDA medium at 28 °C

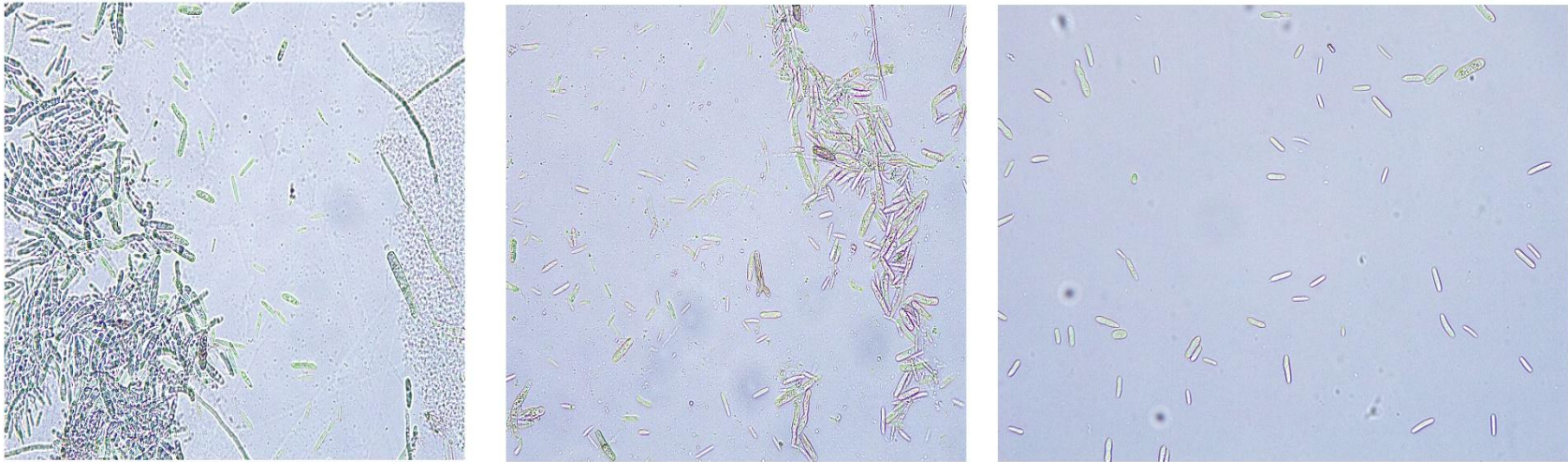


Figure (4): Antagonism between *S.scitamineum* isolates and biocontrol fungi (*Trichoderma viride* and *Macrophomina phaseolina*); the first column represented control of *S.scitamineum* isolates, the second column biocontrol of *S.scitamineum* by *Macrophomina phaseolina*, and the third column showed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

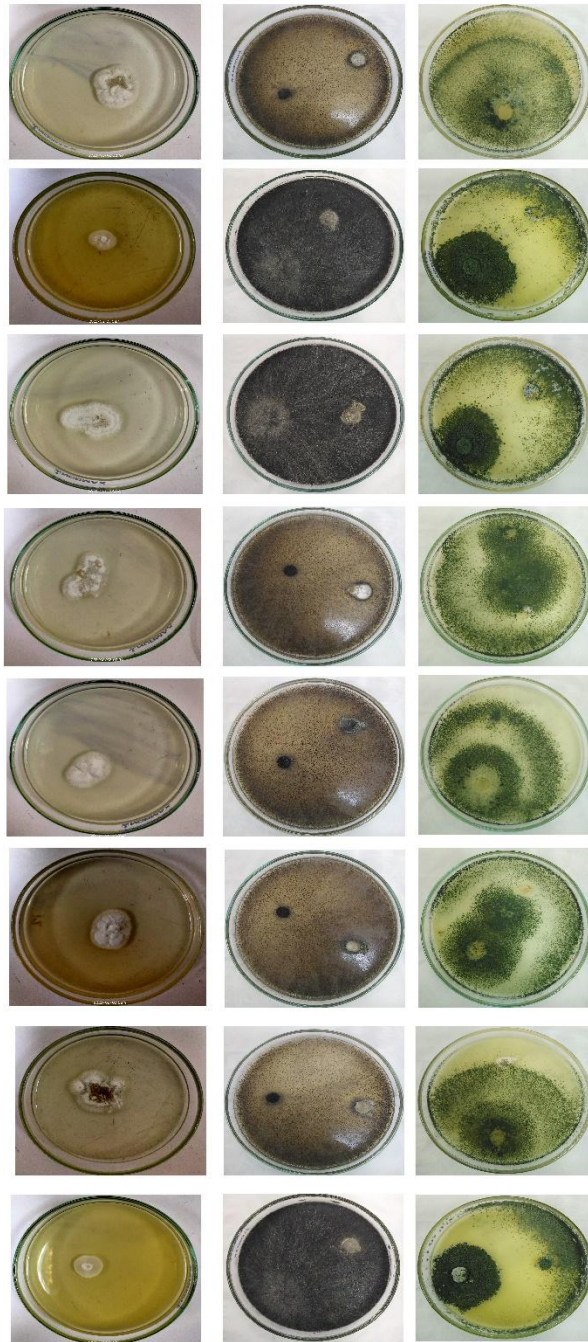


Figure (5): Pathogenicity test on sugarcane inoculated by the pathogen (the left in each image), with the pathogen and biocontrol of *Trichoderma viride* (the right one), and control was the latest image.



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

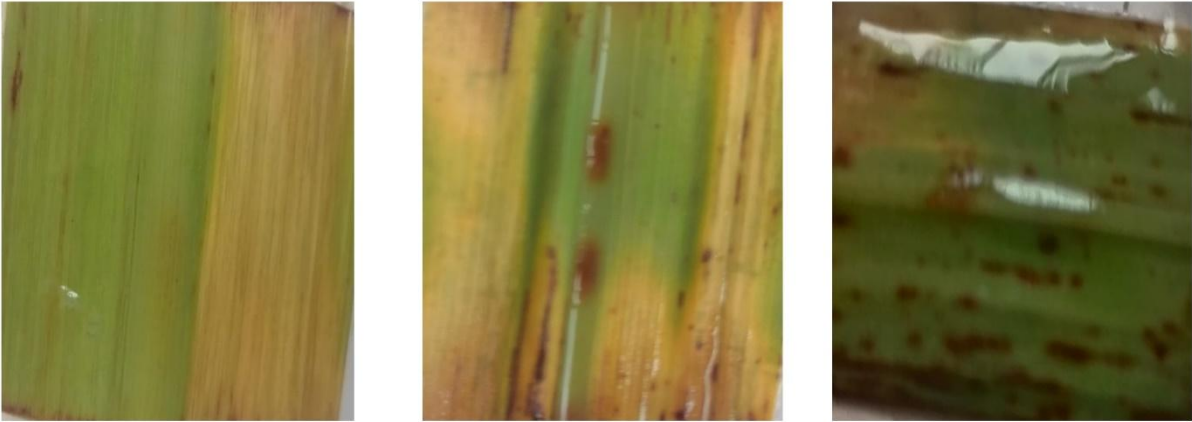


Table (1): Percentage of the growth inhibition % of tested *S. scitamineum* by biocontrol fungi *Trichoderma viride* and *Macrophomina phaseolina*

Isolate number	control	<i>Macrophomina phaseolina</i> (%)	<i>Trichoderma viride</i> (%)
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 <i>S. scitamineum</i> on sugarcane cuttings %	Pathogenicity level of <i>S. scitamineum</i> on sugarcane cuttings inoculated by <i>Trichoderma viride</i> %	Pathogenicity level of <i>S. scitamineum</i> on sugarcane leaves	Diameter of <i>S. scitamineum</i> teliospores	<i>S. scitamineum</i> Sporidia (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%.