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STUDY ON THE ASSOCIATION OF DIFFERENT FUNGAL MICROFLORA IN CASSAVA

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Abstract

The experiments were conducted in field and in the Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh to find out the different diseases of cassava. Here, plant infection, leaf infection and leaf area diseased were found in 75.23%, 18.27% and 26.64%, respectively but low in Crop Botany Field Laboratory, BAU, Mymensingh that was 58.61%, 16.95% and 24.07%, respectively. On the other hand, in rotten tubers the identified organisms were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium spp.* and *Penicillium spp*. which were identified by blotter and PDA method. Both in blotter and PDA method, the incidence was 100.00 % but severity was 48.84% and 55.23%, respectively. In blotter laboratory method, only the *Fusarium spp.* was identified that was 48.84%. In addition, in the PDA method, the severity of the organisms was highest frequency in *A. niger* (66.67%) than medium high frequency was *A. flavus* (10.00%), medium frequency of organism was *Fusarium spp* (16.67%) and small frequency of organism was *Penicillium spp* (6.67%) were recorded under storage condition.

Keywords: Fungal microflora, PDA and blotter methods, as well as cassava

1. Introduction

Cassava, *Manihot esculents* Crantz (Euphorbiaceae) is one of the most important starchy root crop among the tropical food crops (Cock, 1985). Cassava will be appeared one of the earliest crops that have been domesticated and was widespread throughout the new world tropics by the late fifteenth century (FAO, 1995). Growing food deficit is a great concern to all who are interested in the welfare of millions of people who's living under poverty level in Bangladesh.

Attempts to resolve the problems of food production have placed great emphasis on increasing the production and productivity of grain/cereal crops but little attention has been given to crops such as cassava which are produced mainly in the tropics.

Cassava constitutes the principal carbohydrate source for more than 800 million people in developing countries. About 80% of the cassava was consumed by human, while the remaining 20% was used for an agro-industrial purposes as animal feeds (McCann, 1976; IITA, 1992). Cassava is grown under very broad climatic and edaphic conditions.

Increasing world population in some countries has prompted a recent surge of interest in cassava not only for its traditional forms as human food and for specialized starches but also for animal feed stuffs and other industrial uses. This crop has been produced as food crop for more than 500 million people in tropical and sub-tropical Africa, Asia and Latin America (EI-Sharkawy, 2004). Total world production was estimated as 155.3 million tons of dry root weight (FAO, 1993). The leading cassava producing countries in Africa includes, Zaire, Nigeria, Mozambique, Angola, Tanzania, Uganda, Central African Republic, Madagascar, Ghana and Ivory Coast (FAO, 1991). Cassava is grown on around 17 million hectares with an annual yield of 172 million tons. More than 50% of cassava produced is grown in Africa with an annual yield of 91 million tons, 48.2 million tones are produced in Asia and 31.6 million tons in North and South America. In Brazil, the annual production of cassava was 23.3 million tons, and the average yield per hectare was 13.5 tons (Fukuda, 2002). In northern Brazil, most of the cassava was used for human consumption, but in the Central, Western and Southern areas it was mainly used in the manufacture of textiles, paper, glue, medicine and food (Fukuda, 2002).

Plants are completely domesticated and show a high degree of local adaptation mainly in tropical countries. Cassava is a perennial crop and is mostly propagated by woody stem cuttings (CIAT, 1983; IITA, 1990). The large swollen true roots may be harvested 7 months after planting in warm areas, the multiplication of cassava through true seed is becoming important because of increasing breeding research for resistance to major diseases and other agronomic characters, which requires parent true seeds. On dry matter basis, cassava roots contain 92.5% carbohydrate and 3.2% protein; starch and sugar predominate, comprising about 90% (Kawano, 1978; Hahn et al., 1989a). The leaves contain 7% protein on a fresh weight basis and 20-35% on a dry weight basis (IITA, 1992). The protein quality of cassava leaves compare to soybeans are considerably higher in lysine, although deficient in methionine and tryptophane (Jalloh and Dahniga, 1994).

There are many constraints for low production of cassava in which diseases are a great problem. Among the diseases bacterial, fungal and viral are important. Cassava is grown at small scale in Bangladesh. Cassava is attacked by huge number of diseases such as bacterial diseases (Bacterial blight: *Xanthomonas campestris* pv. *manihotis*, Bacterial angular leaf spot: *Xanthomonas campestris* pv. *cassava*, Bacterial wilt: *Erwinia herbicola*); fungal diseases (Anthracnose: *Colletotrichum gloeosporioides f.sp. manihotis,* Blight leaf spot: *Cercospora vicosae*, Cassava ash: *Oidium manihotis*, Fusarium root rot: *Fusarium Oxysporum*/*solani*, Phytophthora root rot: *Phytophthora cryptoge*, Pythium root rot: *Pythium spp*., Sclerotium root rot: *Sclerotium rolfsii*, Verticillium root and stem rot: *Verticillium dahliae*; virus diseases (African cassava mosaic: African cassava mosaic virus, Cassava common mosaic: Cassava common mosaic virus, Cassava common mosaic: Cassava vein mosaic virus, Indian cassava mosaic: Indian cassava mosaic virus). Accurate assessment of yield loss has not been quantified especially due to the problem of multiple disease complexes in Bangladesh. On the view point of the above facts the following objective has been undertaken to study the association of fungal flora with cassava tubers.

Key words: Cassava, Fungal microflora, PDA and Blotter methods.

2. Materials and Methods

2.1. Collection of cassava roots

Cassava roots were collected from the Crop Botany Field Laboratory, Bangladesh Agricultural University (BAU), Mymensingh belonging to the Crop Botany Department, BAU, Mymensingh-2202 for identification of storage and tuber diseases of cassava.

2.2. Isolation, Purification and identification of different fungal microflora associated with Cassava tubers

The experiment was carried out in the MS laboratory of the Plant Pathology Department and Seed Pathology Center (SPC), BAU, Mymensingh.

2.1.2. Isolation

Isolation of the pathogens was made in two ways:

a) By moist blotter method and

b) In potato dextrose agar (PDA) medium.

a) Blotter method

Sterile blotting papers were placed in the petridishes. The petridiches were also surface sterilized by methylated sprit earlier. The diseased plant parts were cut into pieces and placed on

the blotter where moist condition was made by wetting the blotting paper with sterile water. The petridishes were then kept for incubation for the growth and sporulation of the fungi. The sporulating fungi which grew on the wet blotters were identified by a stereoscopic microscope.

b) PDA medium

Twenty four number of PDA plates were made and small pieces of inocula were prepared from the diseased plant parts. The inocula were surface sterilized by dipping them in 10% chlorox for 1.5 minutes. Then, the inocula were washed three times with sterile water. Inocula, thus sterilized were placed aseptically in petridiches containing PDA medium with the help of a pair of sterile forceps. The plates were incubated at 28^0C±1 for several days. The plates were examined daily for fungal growth on the medium. The occurrence of different fungi associated with tubers diseased has been studied.

2.1.3. Purification

The fungi which grew on the medium were transferred to fresh PDA plates from where sub-cultures were made by transferring the block of fungal colony (1mm dia.).

2.1.4. Identification

The fungi were roughly identified by observing colony characters, linear growth, colour and sporulation with the help of CMI description of pathogenic fungi and bacteria and the genus *Fusurium.* Commonwealth Mycol. Inst. Kew, Survey, England. 236p (Anonymous, 1976; Booth, 1971; Ellis, 1971). Microscopic slides with the fungi were made and studied for final conformation. Photographs of the microscopic slides were also taken.

3. Data Analysis

The data were statistically analyzed and the mean values were presented and interpreted for discussion.

4. Results

Incidence and severity of tuber disease / rot of cassava

The Incidence and severity of tuber diseases / rot of cassava were studied in Laboratory using PDA and Blotter method. According to PDA, 100.00% disease incidence was recorded in all the samples. In case of disease severity, the percentage was 58.25, 70.17, 56.90 and 35.59 in sample 1, sample 11, sample III and sample IV, respectively. The incidence and severity of cassava tuber diseases has been shown in Figure 1.

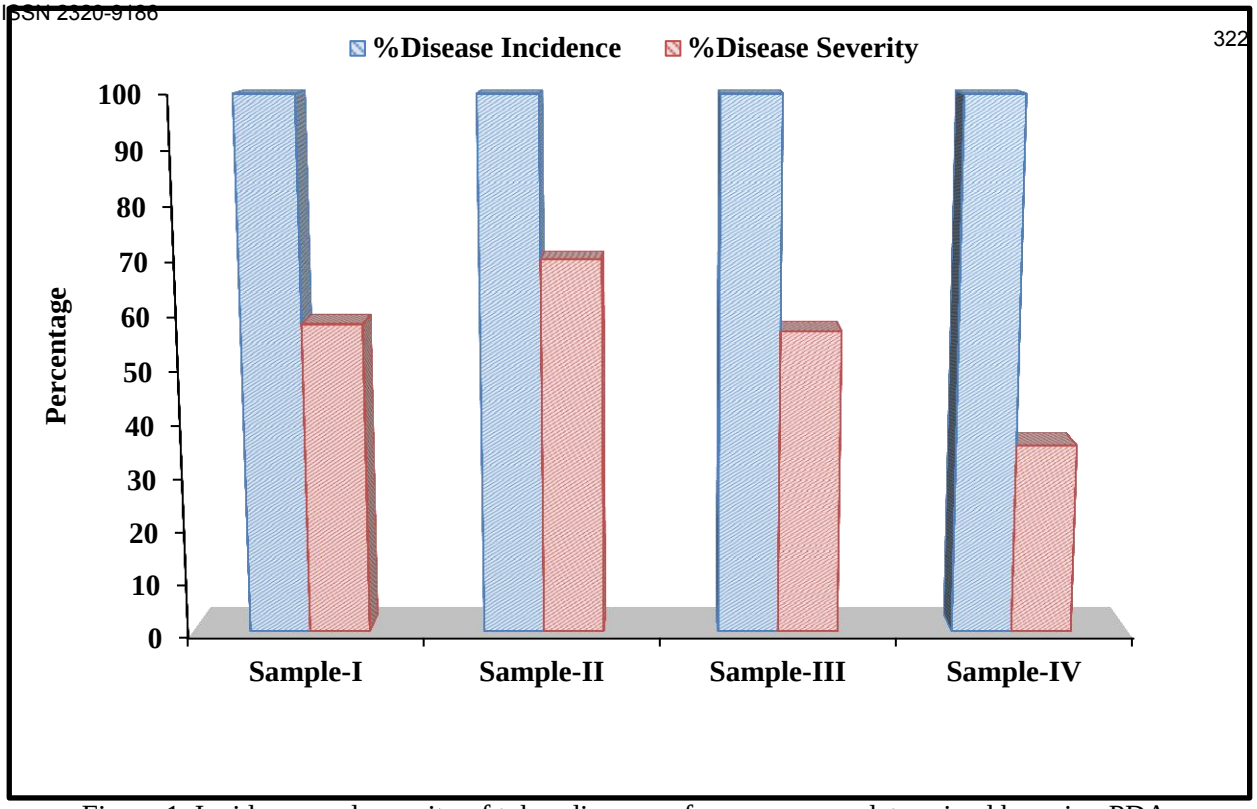

Figure 1. Incidence and severity of tuber diseases of cassava were determined by using PDA

In case of Blotter method, disease incidence was also 100.00% in all the samples. So far as disease severity is concerned, 58.85% and 38.82% was recorded in samples I and samples II, respectively. The incidence and severity of cassava tuber diseases has been shown in Figure 2.

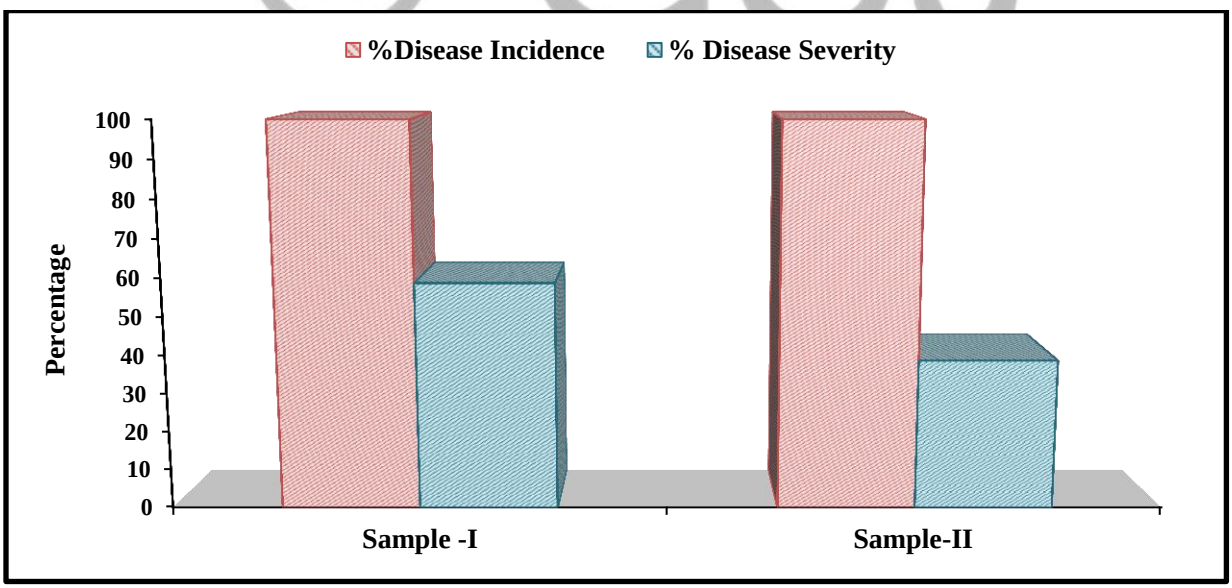

Figure 2. Incidence and severity of tuber diseases of cassava were determined by blotter method

In PDA, the association of three types of pathogen were found in sample I and they were *Aspergillus* spp. (*Aspergillus niger*, *Aspergillus flavus*), *Fusarium spp.* and *Penicillium spp* (Figure 3).

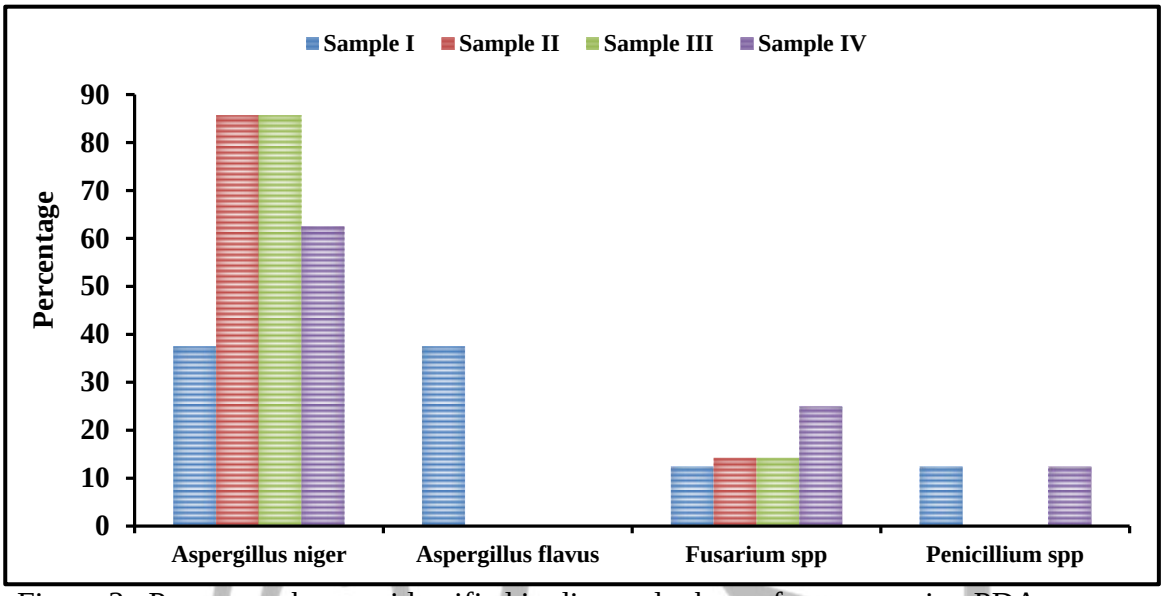

Figure 3. Percent pathogens identified in diseased tubers of cassava using PDA

Aspergillus niger, *Aspergillus flavus*, *Fusarium spp.* and *Penicillium spp*. were recorded using PDA and the mean percent of these organisms were 66.67 %, 10.00 %, 16.67% and 6.67%, respectively which has been shown in Figure 3.

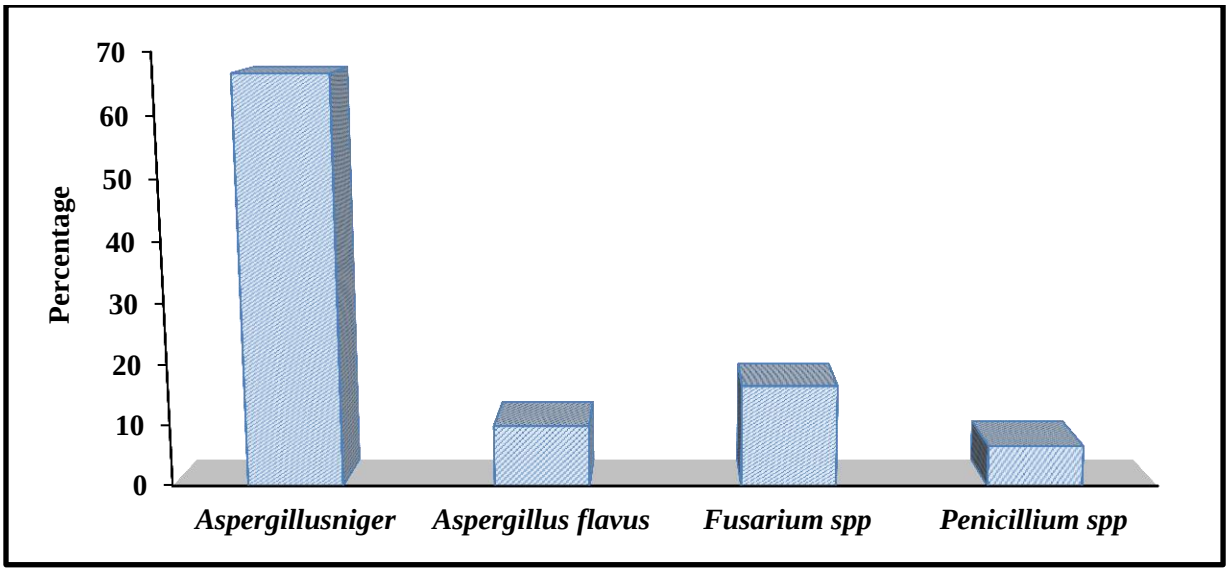

Figure 4. Percent occurrence of pathogens in or with the diseased tubers of cassava determined by PDA

In Blotter method only the *Fusarium spp* was identified 48.84 %. The incidence and severity of tuber diseases of cassava both in Blotter and PDA method have been shown in Figure 5.

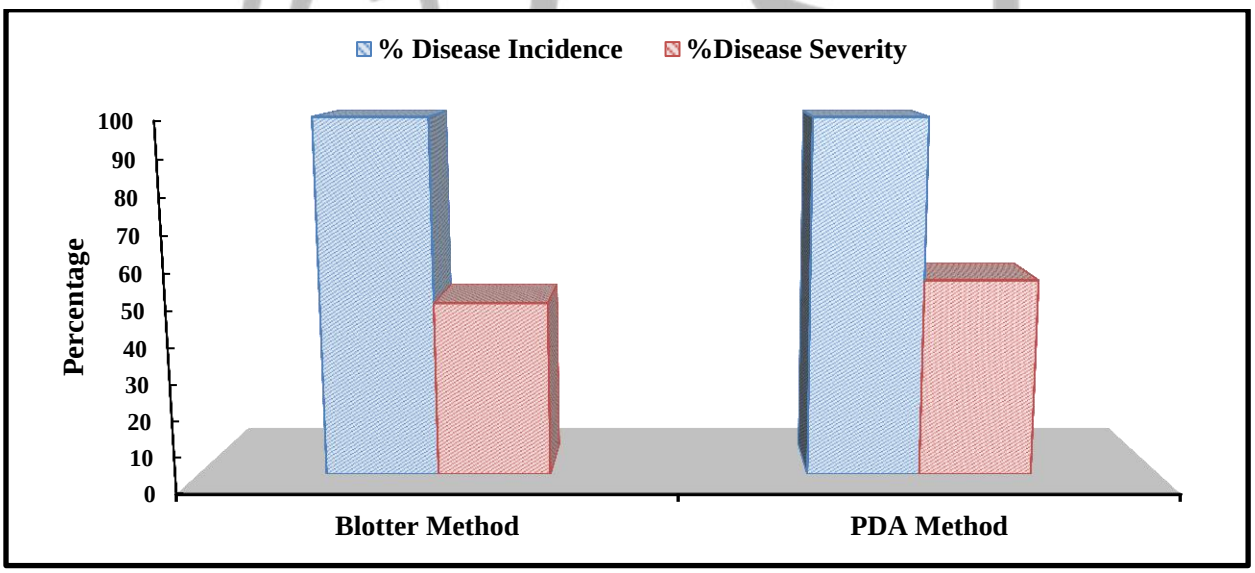

Figure 5. Incidence and severity of tuber diseases of Cassava determined by Blotter and PDA method

5. Discussion

In the Laboratory study, 100% disease incidence was recorded both in PDA and Blotter method. But difference was observed in case of disease severity. Here in PDA, the disease severity was 58.25%, 70.17%, 59.90% and 35.59%, in sample I, sample II, sample III and sample IV, respectively. In blotter method, the disease severity was found 58.85% and 38.82% in sample I and sample II, respectively. Isolation of different microflora from diseased cassava tubers were also studied in the Laboratory. *A. niger, A. flavus, Fusarium spp* and *Penicillium spp* were identified out of diseased cassava tubers. This result was in accordance with the findings of Burton (1970), Booth, R.H. et al., (1976), Noon and Booth (1977), Baniqued and Sajise (1988) and Krauss et al. (2000). In PDA medium, the mean percentage of pathogens was 66.67%, 10.00%, 16.67% and 6.67% for *A. niger, A. flavus, Fusarium spp* and *Penicillium spp,* respectively. But only one pathogen namely *Fusarium spp* was identified by blotter method. In PDA, the association of three types of pathogen were found in sample I and they were *Aspergillus spp,* (*A. niger, A. flavus*), *Fusarium spp* and *Penicillium spp*. The highest record was 37.50% that was found in *A. niger* and *A. flavus,* and the lowest percentage was 12.50% found in *Fusarium spp* and *Penicillium spp.* In sample II, two types of pathogen were found namely *A. niger* and *Fusarium spp.* In *A. niger* highest record was found that was 85.70% and lowest record was found in *Fusarium spp* that was 14.28%. In sample III, same pathogen and same percentage were found. Finally in sample IV, three types of pathogen were found namely *A. niger, Fusarium spp* and *Penicillium spp*. The pathogens were found 62.50%, 25.00%, and 12.50% in *A. niger*, *Fusarium spp* and *Penicillium spp*, respectively. In PDA method, the average number of pathogens were 66.67%, 16.67%, 10.00% and 6.67% in *A. niger, A, flavus, Fusarium spp* and *penicillium spp,* respectively. But in Blotter method, only one pathogen namely fusarium *spp* was found.

On the above discussion it is evident that in case of PDA method, *Aspergillus niger* was associated with the highest tank in infected and rotten tubers and was followed by *Fusarium spp, A. flavus* and *Penicillium spp*. Only *Fusarium spp* was detected by the blotter method. On the basis of the fungi grown on PDA medium was found effective than grown on blotter method. Further research need to be carried out to a detailed study of cassava diseases in other cassava growing areas in Bangladesh.

6. Conclusion

The identified organisms were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium spp* and *Penicillium spp*. which were recorded under the laboratory condition by blotter method and potato dextrose agar (PDA). In blotter method, the incidence and severity were observed 100.00 % and 48.84 %, respectively. On the other hand, in PDA method, the incidence and severity were found 100.00% and 55.23%, respectively. In case of using blotter method, only the *fusarium spp* was identified 48.84% while the severity of the organisms was highest in the PDA and the frequency of *A. niger* was 66.67%. Lowest frequency of *Penicillium* was 6.67% followed by *A. flavus* (10.00%) and *Fusarium* (16.67%), respectively.

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